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CONTENTS

No. 1. July, 1947

The Oxygen Content of Arterial Blood in Dogs Breathing Air at Low Barometric Pressures. *Richard A. Miller, Beatrice Stevens Heagan and C. Bruce Taylor* 1
Circulatory and Cerebral Changes and Protective Aids During Exposure to Acceleratory Forces. *S. W. Britton, V. A. Pertzoff, C. R. French and R. F. Kline* 7
The Distribution of Total Electrolyte, Potassium and Sodium in the Cerebral Cortex in Relation to Experimental Convulsions. *Harry F. Colfer and Hiram E. Essex* 27
The Action of Adrenaline on Spinal Neurones Sensitized by Partial Isolation. *George W. Stavraky* 37
The Action of Insulin as Indicated by Depancreatized Herbivora. *P. O. Greeley* 46
Further Studies on Streamline Blood Flow in the Arteries of the Cat. *H. J. Ralston, A. N. Taylor and H. N. Elliott* 52
Stability of Prothrombin. *Arnold G. Ware, M. Mason Guest and Walter H. Seegers* 58
The Effect of Discontinuous Chronic Anoxia on Liver Glycogen Stores. *Orr E. Reynolds* 65
Relationship between Body Temperature and Blood Sugar in the Chicken. *S. Rodbard* 67
Measurement of the Respiratory Volumes of Laboratory Animals. *Arthur C. Guyton* .. 70
Analysis of Respiratory Patterns in Laboratory Animals. *Arthur C. Guyton* 78
Pituitary and Ovarian Dysfunction in Experimental Diabetes. *Elva G. Shipley and Katherine S. Danley* 84
A Quantitative Study of the Effect of Hyperthyroidism on Genital Structure and Function. *Kenneth M. Richter and Charles A. Winter* 95
Acclimatization to Extreme Cold. *Steven M. Horvath, A. Freedman and H. Golden* 99
The Fatigue of Standing. *Eleanor M. Larsen* 109
Relations between Cutaneous Blood Flow and Blood Content in the Finger Pad, Forearm, and Forehead. *A. B. Hertzman, W. C. Randall and K. E. Jochim* 122
Studies on the Recurrence of Decompression Sickness on Re-ascent to High Altitudes. *S. Rodbard* 133
The Effect of Oxygen, Altitude and Exercise on Breath-Holding Time. *S. Rodbard* 142
The Effect of Stimulation of the Carotid Sinus Region on Absorption of Chloride from the Small Intestine. *Edward J. Van Liere, J. Clifford Stickney and David W. Northup* 149
The Size and Function of the Human Heart at Rest in Semi-Starvation and in Subsequent Rehabilitation. *Ancel Keys, Austin Henschel and Henry Longstreet Taylor*. 153
Plasma Volume and Thiocyanate Space in Famine Edema and Recovery. *Austin Henschel, Olaf Mickelsen, Henry Longstreet Taylor and Ancel Keys* 170
The Effect of Intra-Arterial Injection of Adrenalin upon Blood Flow of the Human Forearm. *Karl Harpuder, Jacob Byer and Irwin D. Stein* 181
Renin, Hypertensinogen, and Hypertensinase Concentration of Blood of Dogs During the Development of Hypertension by Constriction of the Renal Artery. *Florence W. Haynes and Lewis Dexter* 190
Renin Content of Renal Venous Blood of Normal and Hypertensive Patients at Rest. *Florence W. Haynes, Lewis Dexter and Roy E. Seibel* 198
Alveolar Air During Simulated Flights to High Altitudes. *Hermann Rahn and Arthur B. Otis* 202
Retention of Normal Insulin Tolerance and Adrenal Cortex after Extirpation of the Hypophysial Stalk in the Dog. *Allen D. Keller and Carlyle G. Breckenridge* 222

No. 2. August, 1947

Quantitative Studies of Chronic Facilitation in Human Motoneuron Pools. *J. S. Denslow, Irvin M. Korr and A. D. Krems* 229

Blood-Borne Vasotropic Substances in Experimental Shock. *Robert Chambers and B. W. Zweifach* 239
The Effect of Amphetamine Sulfate and Some Barbiturates on the Fatigue Produced by Prolonged Wakefulness. *David B. Tyler* 253
Blood Sugar Levels and the Behavior Pattern of Young Healthy Adults Several Hours after the Ingestion of Large Amounts of Sucrose. *John Haldi and Winfrey Wynn*. 263
Recording of Blood Pressure from the Left Auricle and the Pulmonary Veins in Human Subjects with Interauricular Septal Defect. *A. Cournand, H. L. Motley, A. Himmelstein, D. Dresdale and J. Baldwin* 267
An Analysis of Changes in the Contour of the Femoral Arterial Pulse During Hemorrhagic Shock. *Robert S. Alexander and Edward A. Webb* 272
The Evaluation of the Work of the Heart. *J. W. Remington and W. F. Hamilton* 292
The Rate of Removal of Intravenously Injected Bromsulphalein by the Liver and Extra Hepatic Tissues of the Dog. *Clarence Cohn, Rachmiel Levine and Daniel Streicher*. 299
Control of Peripheral Blood Flow: Responses in the Human Hand when Extremities are Warmed. *B. G. Ferris, Jr., R. E. Forster, II, E. L. Pillion and W. R. Christensen*.. 304
The Influence of Short Periods of Induced Acute Anoxia upon Pulmonary Artery Pressures in Man. *Hurley L. Motley, Andre Cournand, Lars Werko, Aaron Himmelstein and David Dresdale* 315
The Influence of Adrenalin on Blood Sugar and Resistance to Hypoxia in the Nembutalized Cat. *Robert H. Oster and Dietrich C. Smith* 321
Modification of the Action Potential of Amphibian Nerves by Triturus Embryonic Toxin. *R. S. Turner and F. A. Fuhrman* 325
The Effect of Environmental Temperature on Food Selection. *Sz. Donhoffer and J. Vonotzky* 329
The Effect of Thyroxine on Food Intake and Selection. *Sz. Donhoffer and J. Vonotzky*. 334
The Clearance of Inulin and Sodium P-Aminohippurate in the Rat. *Sydney M. Friedman, John R. Polley and Constance L. Friedman* 340
Recovery of a Pressor Principle from the Blood Plasma of Cats Given Kidney Extracts. *O. M. Helmer and R. E. Shipley* 353
The Carotid-Mandibular Reflex in Acute Respiratory Failure. *Robert D. Tschirgi and R. W. Gerard* 358
Local Sweat Gland Activity Due to Direct Effects of Radiant Heat. *Walter C. Randall*. 365

No. 3. September, 1947

Alterations in Urogastrone Excretion Produced by Extirpation of Various Endocrine Glands. *J. Kaulbersz, T. L. Patterson, D. J. Sandweiss and H. C. Saltzstein* 373
The Plasmatic Cofactor of Thromboplastin: Its Adsorption, with Prothrombin and Fibrinogen, by Alumina and Tricalcium Phosphate Gels. *René Honorato* 381
The Effect of Carbohydrate Feeding on the Output of Urinary Amino Acid. *Anthony A. Albanese, Virginia Irby, Jane E. Frankston and Marilyn Lein* 389
The Induction of Lactation During Pregnancy in Rabbits and the Specificity of the Lactogenic Hormone. *Joseph Meites and C. W. Turner* 394
The Effects of the Pituitary Growth and Adrenocorticotropic Hormones on the Urinary Glucose and Nitrogen of Diabetic Rats. *Leslie L. Bennett and Choh Hao Li* 400
The Relation of Fibrinogen to the Coagulation Factor Which Diminished in Stored Plasma. *René Honorato and Armand J. Quick* 405
The Reversible Inactivation of Prothrombin: A Factor Responsible for its Partial Reactivation. *Muriel Platt Munro and F. L. Munro* 409
Disappearance of Uropepsin from the Urine of Gastrectomized Cats. *Gladys R. Bucher and A. C. Ivy* 415
The Alleged Excretion of Histamine in Parathyroidectomized Dogs. *G. Misrahy and S. Salama* 420

Effect of Adrenal Cortex Extract upon the Tolerance of the Eviscerated Rat for Intravenously Administered Glucose. *Dwight J. Ingle, Mildred C. Prestrud, James E. Nezamis and Marvin H. Kuizenga* 423
Comparative Effectiveness of Albumin, Globin, Hemoglobin, Gelatin, Oxopolygelatin, Saline, Ringer's, Blood and Plasma upon the Survival of Rats Subjected to Standardized Scald Burns. *M. D. McCarthy and W. M. Parkins* 428
Bioassay of Steroid Hormones Using Aqueous Sodium Lauryl Sulfate Solution as the Dispersing Agent. *Fritz Bischoff and Harry R. Pilhorn* 444
Observations on the Action Potentials Induced by Indirect Stimulation of Skeletal Muscle in Desoxycorticosterone Acetate-Treated Rats on a Low Potassium Diet. *Sheppard M. Walker* 451
The Effect of Dietary Fat upon Gastric Evacuation in Normal Subjects. *J. H. Annegers and A. C. Ivy* 461
Systemic Blood Pressure as a Factor in the Absorption of Saline from the Small Intestine. *J. Clifford Stickney, David W. Northup and Edward J. van Liere* 466
Physiological Effects of a Plasma Protein: Blood Pressure, Leucocyte Concentration, Smooth and Cardiac Muscle Activity. *M. Mason Guest, Robert C. Murphy, Stephen R. Bodnar, Arnold G. Ware and Walter H. Seegers* 471
Adrenal Response to Total Body X-Radiation. *H. M. Patt, M. N. Swift, E. B. Tyree and E. S. John* 480
An Experimental Study of Intramuscular Pressure Measurements. *Campbell Moses* .. 488
The Effects of Anoxemic Anoxia on Excitability, Conduction and Refractoriness of Mammalian Cardiac Muscle. *A Sidney Harris and Wilson P. Matlock* 493
The Use of Acetylcholine in the Objective Determination of Circulation Time and the Fractionation of the Vascular Bed Traversed. *M. Wilburne, J. G. Schlichter, M. Grossman and F. Cisneros* 504
The Effects of Dietary Caloric Restriction on Maturity and Senescence, with Particular Reference to Fertility and Longevity. *Zelda B. Ball, Richard H. Barnes and Maurice B. Visscher* 511
The Effect of Adding Boron to a Potassium-Deficient Diet in the Rat. *Richard H. Follis, Jr* 520

No. 4. October, 1947

An Examination of Some Factors which Alter Glomerular Activity in the Rabbit Kidney. *Roy P. Forster* 523
Effect of Experimental Neurogenic Hypertension on Renal Blood Flow and Glomerular Filtration Rates in Intact Denervated Kidneys of Unanesthetized Rabbits with Adrenal Glands Demedullated. *Roy P. Forster and Julian P. Maes* 534
Effect of Ether and Pentobarbital on the Polarisation State of Central Nervous Elements. *A. van Harreveld* 541
Effects of Vitamin Imbalance Under Conditions of Ad Libitum Feeding and Reduced Caloric Intake. *Virginia H. Brunish, H. B. McWilliams, G. D. Mason, A. D. Adams, Jr. and B. H. Ershoff* 551
Electromyographic Studies on Cats After Section and Suture of the Sciatic Nerve. *James G. Golseth and James A. Fizzell* 558
Refractive Changes Produced by Injection of Fluids into the Vitreous Humor. *Arthur Layton, Meredith W. Morgan, Jr. and J. M. D. Olmsted* 568
Electrical Characteristics of Injuries to Heart Muscle. *J. A. E. Eyster and W. E. Gilson* 572
The Effect of Adrenalectomy, Adrenal Cortical Hormones, and Testosterone Propionate Plus Adrenal Cortical Extract on the "Alkaline" and "Acid" Phosphatases of the Liver and Kidney of the Rat. *Virginia N. Vail and Charles D. Kochakian* 580
Hyperglycemia Induced by the Action of Adrenalin on the Central Nervous System. *A. Leimdorfer, R. Arana and M. H. Hack* 588

Relative Activity of Wrist Moving Muscles in Static Support of the Wrist Joint: An Electromyographic Study. *Wilfrid T. Dempster and John C. Finerty* 596
An Experimental Study of Explosive Decompression Injury. *E. L. Corey* 607
The Effect of Alloxan on Muscle Glycolysis. *Chalmers L. Gemmill* 613
The Relation of O_2 in Bone Marrow Blood to Post-Hemorrhagic Erythropoiesis. *Wilson C. Grant and Walter S. Root* 618
Influence of Environmental Temperature and Posture on Volume and Composition of Blood. *C. R. Spealman, Michael Newton and R. L. Post* 628
A Comparison of Ten Infusion Fluids in the Treatment of Moderate and Severe Hemorrhage in Animals. *Angie S. Hamilton, William M. Parkins and Frederic Waltzer* 641
The Effect of Pulmonary Vascular Congestion on the Distensibility of the Lungs. *I. Mack, M. Grossman and L. N. Katz* 654
A Quantitative Study of Antifibrinolysin in Chick Plasma: Increase in Antifibrinolysin Activity During Pteroylglutamic Acid Deficiency. *M. Mason Guest, Arnold G. Ware and Walter H. Seegers* 661
On the Mechanism of the "Spontaneous" Re-Innervation in Paretic Muscles. *A. van Harreveld* 670
The Blood Uric Acid and Allantoin of the Rat After Nephrectomy and Hepatectomy. *Sanford O. Byers, Meyer Friedman and Morton M. Garfield* 677
The Effect of Insulin upon the Level of Blood Amino Acids in the Eviscerated Rat as Related to the Level of Blood Glucose. *Dwight J. Ingle, Mildred C. Prestrud and James E. Nezamis* 682
The Thyroid Secretion Rate of Growing and Mature Mice. *Victor Hurst and C. W. Turner* 686
Ischemic Compression Shock: Influence of Body Temperature and of Temperature of Traumatized Tissues. *Edgar L. Lipton, Adam B. Denison and Harold D. Green*... 693
Effect of Heparin on Ischemic Compression Shock. *J. Richard R. Bobb and Harold D. Green* 697
Rôle of the Kidney in Resistance to Ischemic Compression Shock. *J. Richard R. Bobb and Harold D. Green* 700
Studies on the Temperature Characteristics, Blood Flow and Activity in Normal and Denervated Limbs of the Dog. *C. R. Kemp, W. W. Tuttle and H. M. Hines* 705
An Analysis of the Carotid Sinus Cardiovascular Reflex Mechanism. *S. C. Wang and Herbert L. Borison* 712
Decussation of the Pathways in the Carotid Sinus Cardiovascular Reflex: an Example of the Principle of Convergence. *S. C. Wang and Herbert L. Borison* 722
The Change in Specific Gravity of the Blood Plasma of the Rat During Severe Water Privation. *Paul S. Siegel, Irving E. Alexander and Helen L. Stuckey* 729
On the Energy-Rich Phosphate Supply of the Failing Heart. *Albert Wollenberger*.... 733
Distribution of Esterase in Lymph from Various Regions and in Relation to Lymphoid Tissue. *Ralph W. Brauer and Esther Hardenbergh* 746
The Use of the Shay Rat in Studying Anti-Ulcer Substances. *E. A. Risley, W. B. Raymond and R. H. Barnes* 754
Effect of Level of Thyroid Activity on Response of Ovariectomized Rats to Estrone. *Wright Langham and R. B. Gustavson* 760
Index 769

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THE OXYGEN CONTENT OF ARTERIAL BLOOD IN DOGS BREATHING AIR AT LOW BAROMETRIC PRESSURES

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It has been observed repeatedly in this laboratory that dogs are quite resistant to anoxia resulting from exposure to low barometric pressure. They remain active and conscious during considerable periods of exposure to pressures at which men quickly become unconscious. Obviously dogs either remain conscious in spite of a low arterial oxygen saturation or they compensate more completely for low tensions of oxygen in inspired air and maintain a higher oxygen saturation of the blood than does man. To distinguish between these possibilities a series of dogs were exposed to low barometric pressures in a decompression chamber and the oxygen saturation of the blood determined.

METHODS. Unanesthetized healthy dogs breathing air were used. Blood samples were drawn at ground level and at altitudes between 5000 and 35,000 feet from the femoral artery into syringes coated with paraffin oil. Heparin was the anticoagulant. Duplicate analyses of each sample of whole blood for oxygen and carbon dioxide were made according to the manometric method of Van Slyke and Neill (11). The oxygen capacity was estimated in blood equilibrated with atmospheric air in a tonometer in a water bath at 20°C. All estimations were corrected to exclude oxygen in physical solution (8).

The dogs were decompressed at a simulated rate of ascent of 3000 feet per minute in large well ventilated decompression chambers. Barometric pressure was measured with a mercury manometer and is reported as simulated altitude (hereafter referred to simply as altitude) according the the U. S. Standard Atmosphere Table. In about a third of the experiments two samples of blood were drawn from each dog, one at ground level (750 ft.) immediately before ascent and the other at altitude. In the other experiments blood was drawn only at altitude. The interval at maximum altitude before blood was taken varied from 16 to 49 minutes.

RESULTS. Thirteen estimations of the oxygen saturation and carbon dioxide

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content of arterial blood drawn from seven dogs at ground level were made (table 1). The oxygen saturation ranged from 86 to 99 percent, the average being 95.26 percent. In ten of the thirteen estimations the oxygen saturation was 95 percent or more. One sample was 86 percent saturated, the other two between 90 and 95 percent.

The carbon dioxide content of whole blood in volumes percent varied from 25 to 47 with an average of 35.47. Two thirds of the estimations were between 31 and 40.

Twenty-nine samples of blood were collected from eight dogs breathing air at altitudes between 5000 to 35,000 feet (table 2). Most of the points on the curve in figure 1 are an average of three to five determinations made on blood from at least two dogs. At both 18,500 and 28,000 feet two samples of blood were collected.

TABLE 1

Oxygen and carbon dioxide content of arterial blood of dogs at ground level (750 feet)

ANIMAL NUMBER	O_2Hb CONTENT	O_2Hb CAPACITY	SATURATION OF HEMOGLOBIN	CO_2 CONTENT	HEMATOCRIT
	Vol. %	*Vol. %*	*%*	*Vol. %*	*% cells*
1	13.46	13.69	98.31	34.59	33
1	13.74	14.31	96.01		35
1	14.55	15.17	95.91	31.34	38
1	15.84	16.29	97.24	29.98	40
2	19.96	23.13	86.29	46.83	46
2	18.46	19.52	94.57	33.96	45
3	21.38	23.54	90.82	39.64	56
4	23.00	23.33	98.59	36.56	55
4	21.97	23.07	95.24	31.83	56
5	16.83	17.50	96.17	43.89	47
6	13.99	15.53	90.08	35.78	
6	15.80	15.90	99.37	36.30	44
7	19.94	19.97	99.85	24.95	53

The extent of variation in the saturation at each altitude is shown in figure 1. At 35,000 feet one dog, number 7, had a saturation markedly greater than those of the other four animals at that altitude. This one estimation raised the average of the group of five animals above the greatest saturation among the other four. The average saturation excluding this exceptional value was 20.22 percent. Circumstance made it impossible to draw another sample of blood at this same altitude from dog 7.

A straight line appears to be the best fit to the average saturations at altitudes between 5000 and 35,000 feet (fig. 1). Except at 20,000 feet the average of the determinations does not vary from corresponding points on the smoothed curve by more than 4 percent. A greater number of determinations at the several altitudes is, of course, desirable but it does not appear that additional estimations would make a major change in the curve.

At altitudes above 20,000 feet the carbon dioxide content of arterial blood fell

rapidly (table 2). Between 5000 and 20,000 feet the carbon dioxide content was higher than at ground level. The ground level determinations were lower than those Stewart (10) reported for dogs. Since our samples at ground level were drawn while the animals were breathing rapidly in the excitement of being placed upon the operating table, hyperventilation may be responsible for the apparently low carbon dioxide content.

At altitudes below 35,000 feet there was never any question of the dogs not being conscious. Even at 30,000 feet, where the arterial oxygen saturation was 29.5 percent, the dogs were able to walk, reacted to light and sound, and tensed when the arterial puncture was made. After 21 to 30 minutes at 35,000 feet four of the animals were likewise conscious although the blood of one animal was

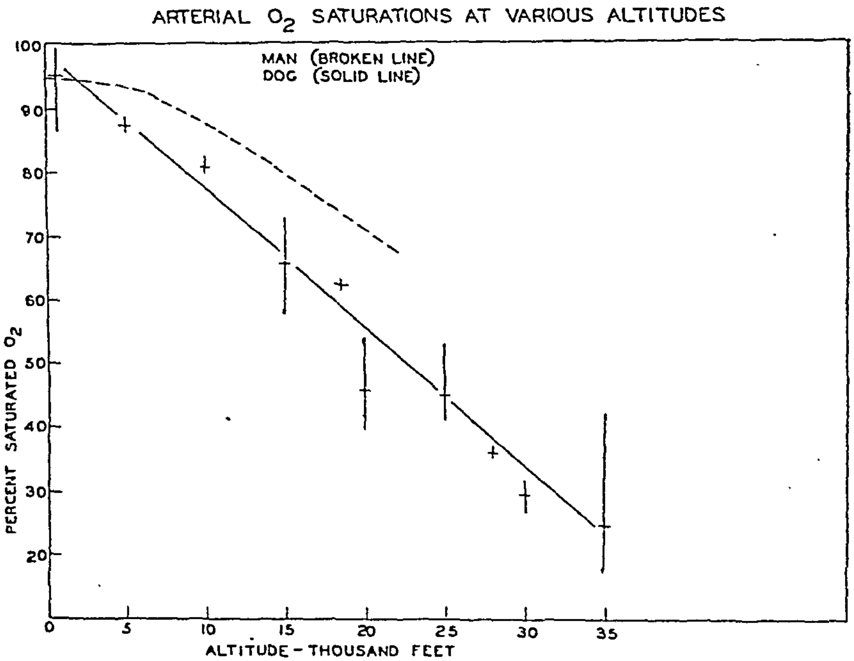

Fig. 1. Arterial O_2 saturations at various altitudes.

only 15 percent saturated. Two of the animals walked about the chamber, two were unable to walk but retained a measure of co-ordination in posture. All four lifted and turned their heads toward noise, reacted when the lights were turned off and on, or winked at a hand waved toward their eyes. Thus although these animals were extremely anoxemic and exhibited marked impairment of muscular co-ordination, it was our opinion that they were not unconscious.

Dog number 2 at 35,000 feet was clearly unconscious. Five minutes after reaching 35,000 feet he was incontinent of urine. The body was quite relaxed and moving him onto the operating table elicited no reaction. He did not respond to sound, light or to the arterial puncture. The arterial oxygen saturation of this animal was 21.8 per cent.

Discussion. Stewart (10) reported the arterial oxygen saturation of a large

group of unanesthetized dogs as ranging between 92 to 95 percent with the greatest number between 92 and 93 percent. If no correction were made for the amount of oxygen in physical solution these percents should be raised some-

TABLE 2

Oxygen and carbon dioxide content of arterial blood of dogs exposed to reduced barometric pressure

ANIMAL NUMBER	SIMULATED ALTITUDE	TIME AT ALTITUDE	O_2Hb CONTENT	O_2Hb CAPACITY	% SATURATION HEMOGLOBIN	CO_2 CONTENT	HEMATOCRIT
	feet	*minutes*	*Vols. %*	*Vol. %*	*%*	*Vol. %*	*% CELLS*
3	5,000	42	17.04	19.48	87.47	44.79	46.5
8		27	17.55	19.79	88.68	39.25	46.0
2		34	15.59	18.10	86.13	35.27	40.0
2	10,000	24	15.55	18.83	82.58	42.48	49.0
3		32	15.99	19.97	80.07	39.25	50.0
8		42	16.34	20.44	79.94	36.27	49.0
2	15,000	35	13.03	19.67	66.24	36.36	48.0
3		39	10.81	18.66	57.88	40.73	46.0
1		20	15.76	21.66	72.76	32.36	48.0
4	18,500	17	14.71	23.30	63.13	32.70	55.0
5		16	9.83	15.95	61.63	36.60	46.0
8	20,000	26	9.23	20.02	46.10	35.97	45.5
3		43	7.53	19.02	39.59	37.05	45.0
3		19	9.21	19.33	47.65	33.46	50.0
2		26	10.61	19.69	53.88	34.53	46.0
3		19	8.52	20.52	41.52	38.53	50.0
4	25,000	49	13.31	25.09	53.05	21.57	59.5
2		30	8.68	21.05	41.23	36.90	46.0
2			8.21	19.97	41.11	38.42	
1	28,000	28	7.56	20.71	36.50	22.80	49.0
2		21	7.31	20.52	35.62	31.56	51.0
1	30,000	25	3.65	13.60	26.84	13.08	38.5
6		22	4.70	15.72	29.90	24.30	43.0
2		23	7.38	23.23	31.77	24.31	55.0
7	35,000	29	8.55	20.12	42.50	9.86	51.5
6		21	3.24	15.73	20.60	15.12	46.5
1		22	2.38	15.81	15.05	3.58	39.0
8		30	5.08	21.66	23.45	17.17	54.0
2		24	4.84	22.20	21.80	16.37	54.0

what. Even so the saturation would still be lower than that found in the present study. Using a spectrophotometric procedure Drabkin and Schmidt (2) reported that blood collected at the end of an inspiration was 98.5 percent saturated. Two samples collected at the end of expiration were 95.5 and 96.6

percent saturated. Recently Roughton et al. (9) have shown that there are several sources of error in the usual Van Slyke procedure by which the arterial oxygen saturation is low by about 2 percent. If this correction is applied to our determinations the value of 97 percent is similar to that reported by Drabkin and Schmidt for dogs and to the value of 97 to 98 percent saturation which is now considered to be the correct value for human blood (2) (9).

Opitz and Tilman (7) made a few estimations of the oxygen content of blood drawn from anesthetized dogs at simulated altitude.. Above 20,000 feet their average saturations were 5 to 14 percent higher than the values determined in the present work. Since the blood was apparently drawn immediately on reaching an altitude the arterial oxygen tension may not have reached equilibrium.

Gray (3) has compiled from several sources the oxygen saturation of arterial blood of men at altitudes up to 22,000 feet. The smoothed curve is reproduced in figure 1. In comparison with these data for man, the arterial oxygen saturation of dogs is from 9 to 16 percent lower. Hemingway (4) found a saturation of 56.6 percent at the moment when the subjects stopped writing at 35,000 feet.

Man has an increasingly shorter period of consciousness above 20,000 feet. At 25,000 feet the period of useful consciousness is about five minutes and decreases to about seventy-five seconds at 35,000 feet (1) (5) (4) (6). In most of these investigations the end point was the moment when the subject stopped writing. Armstrong's (1) subjects lapsed into coma after six minutes at 25,000 feet and one minute at 35,000 feet. In contrast to these observations, dogs still were conscious after exposures for from 19 to 49 minutes at altitudes between 20,000 and 35,000 feet. Thus although anoxemia is more severe in dogs than in man at comparable altitudes, the dog is more resistant to the effect of anoxemia and retains consciousness for longer intervals.

SUMMARY

1. The oxygen and carbon dioxide content of thirteen samples of arterial blood from seven dogs at ground level and of twenty-nine samples drawn from eight dogs after 16 to 49 minutes at simulated altitudes from 5000 to 35,000 feet was measured.

2. The average arterial oxygen at ground level (750 ft.) was 95.26 percent. The average saturation decreased from 87.43 percent at 5000 feet to 24.68 per cent at 35,000 feet. The carbon dioxide content of whole blood fell from 39.77 volumes percent at 5000 feet to 12.42 volumes percent at 35,000 feet.

3. Four of the five dogs at 35,000 feet were judged to be conscious at the time blood samples were drawn; one dog was unconscious.

4. The arterial oxygen saturation and length of consciousness are compared in dogs and in man. Dogs retain consciousness for much longer intervals than does man even though the arterial oxygen saturation of dogs is 9 to 16 percent lower than that of man at altitudes between 5000 and 22,000 feet.

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CIRCULATORY AND CEREBRAL CHANGES AND PROTECTIVE AIDS DURING EXPOSURE TO ACCELERATORY FORCES[1]

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Results derived from experiments carried out mainly on small animals during the early part of the war have recently been reported (Pertzoff and Britton, 1945, 1946; Britton *et al.*, 1946). More particular inquiry into circulatory, cerebral and other changes brought about by high accelerations has been made in the past three years and may now be discussed. It demands no keen insight to visualize the need for more intimate knowledge of functional disturbances referable to acceleratory and deceleratory forces, with the present and immediately anticipated developments in aerial travel. While high velocity motion at uniform speed and direction does not give us physiological concern, bodily changes induced by starting and stopping the physical machinery (perhaps somewhat suddenly now and then), or changing the direction of movement, may be of no small caliber.

Apparatus and methods. The greater part of our work was carried out on a centrifuge with 20-foot heavy steel revolving beam, tapered and fitted at the end (of each 10-foot arm) for carriage of apparatus. Animal holders were attached to the rotors, sometimes inside a low pressure chamber, and manometers for blood pressure and blood flow, movie camera and lights, terminal boxes, etc., were fixed in various positions on the centrifuge. A counter-weight was used on one arm when one animal only (on the opposite arm) was under exposure. Electrical leads for E.C.G., E.E.G., blood pressure, timing, etc., passed back along the centrifuge arm, through concentric rotating mercury pools ("slip-rings") with platinum or copper dip-contacts, and overhead through heavy iron conduits to receiving and amplifying instruments in a separate observation cage. A pipe-line to the pressure chambers on the centrifuge for air evacuation or compression and gas supply passed through a central slip-connection. The character of the experiments demanded great care to avoid electrical and other artifacts and enable optimal detection of minute electropotential changes during centrifugation. Concrete and steel barricades and various electrical devices were employed for personnel safety.

The centrifuge was powered by a 75-h.p., 250 v. D.C. vertical motor with direct drive, capable of developing 120 r.p.m. Current was provided by a 100 h.p. line-starter or motor-generator set, 220 v., 3-phase, 60-cycle; it was housed in a separate building 50 feet from the experimental laboratory, to avoid electrical interference during tests. Controls were located in good view of the centrifuge

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Virginia.

and adjacent to the main recording instruments. Although accelerations up to 6 or 8 g were generally used, 25 or 50 g (up to 120 r.p.m. at 10 ft. radius) could be developed in two or three seconds.

For the determination of most of the arterial pressure readings a mercury manometric system which recorded electrically was devised, somewhat similar to that used by Jongbloed and Noyons (1933). A 50-micron-diameter platinum wire passed through and was fixed in a 1.5 mm.-bore graduated glass tube containing the mercury column; it was then connected, through a pair of mercury slip-rings at the center of the centrifuge, to a modified d'Arsonval-bridge built around a micro-ammeter. Pressures were transmitted to the mercury column *via* the small reservoir at its base through a T-cannula of largest size suitable for the artery (usually between 16–20 gauge), inserted into the blood vessel at its proximal portion towards the heart. The instrument, it may be noted, possessed two significant electrical elements—the mercury column with practically infinite conductance, and the platinum wire with relatively high resistance. Changes in the mercury height were determined by changes in electrical resistance, indicated by the bridge-type meter. The mercury column, placed at a right angle to the direction of the acceleration, was found quite stable in control tests. By calibration of the meter readings with those secured from the ordinary U-tube manometer, values were ascertained in millimeters of Hg (1 mm. = *c.*0.2 ohm). This method, while not ideal, proved highly sensitive and gave fairly reliable results even under the severe conditions imposed by high forces. In later experiments in which arterial pressure was studied, the mercury manometer readings were photographed by "movie" camera during centrifugation. Measurements of blood pressure were made by both closed and open vessel methods.

Comparison of arterial pressure levels under acceleratory exposure was made with pressure readings derived from a hydrodynamic model. The latter consisted of two 150 cc. Ehrlenmeyer flasks, 30 inches apart (about the length of the animals used), to each of which was attached at the base about 10 in. glass tubing of 3.0 mm. bore; the ends of the latter were connected by about 6 in. glass tubing of 0.5 mm. bore, to provide a simple capillary resistance. The closed system was filled to about two-thirds with water under pressure of 120 mm. Hg. A 20-gauge needle, connected with the electrically-recording manometer, was inserted into the rubber tubing at one or the other end of the system. Such a mechanical model, it was thought, might give further insight into arterial pressure changes produced in an animal subjected to centrifugal forces.

For recording arterial blood flow in animals during centrifugation, a flow-meter based on the Venturi principle, somewhat similar to that described by Wagoner and Livingston (1928), was constructed. Tests were made with models of different shapes and sizes before one was eventually adopted. The instrument used was essentially a nearly-circular tube with ends below to connect with the artery, and a central constriction above, streamlined as much as possible to prevent eddies in flow; two outlets above, one placed as near as possible to the constriction, were connected to a graduated, inverted U-tube containing physiological

saline solution stained black. When blood passed through the flow-meter, pressure was built up proximally due to the constriction, and a difference in levels in the graduated manometric tubes was set up; this difference reflected and was proportional to the rate of blood flow. The difference in levels was usually photographed (16 mm. movie film, 8 frames per sec.), or sometimes read directly. Calibration was made by checking the instrument with whole or defibrinated ox blood at a wide range of flows and pressures. The device was readily responsive and showed only a small time lag. It was relatively insensitive to slight vibrations and rugged enough for use on the centrifuge.

EXPERIMENTS. Most of the experiments were performed on monkeys (*Macacus rhesus*), dogs and a few cats; they were usually not anesthetized, since it was clear that only slight discomfort was involved. After a few trials, dogs and monkeys became well accustomed to the setting-up procedures on the centrifuge; the light strapping necessary for stabilization, and application of external leads for E.C.G., E.E.G. and respiration, provoked little disturbance. Animals were frequently quiet throughout most of the tests. While slight movement did not vitiate the records (E.E.G., etc.) derived from an animal, vigorous struggling was of course disturbing and showed on all tracings. In those instances in which minor surgical manipulations were necessary, ether or sometimes amytal and a local anesthetic were given; from 30–60 min. were then allowed for recovery before tests were made. Carotid, brachial and femoral arteries were commonly used, and heparin was necessary.

By far the largest group of experiments (over 80 per cent) were non-lethal, requiring mainly skin electrodes for securing brain, heart and respiratory data. It was striking that animals given perhaps 50–100 exposures of 5–20 sec. each (sometimes 5–10 min. exposures were made) at 1–6 g over a 3 or 4 hour period, once every 10–15 days, survived indefinitely in good condition. During a series of runs, rest intervals of 1–5 min. were allowed, according to the severity of the exposures. Parts of typical runs are shown in figure 1. Further technical details have been reported (Britton *et al.*, 1946).

RESULTS. *Heart rate.* An animal under positive g forces displayed quick acceleration of heart rate. This sensitive reaction showed within narrow limits a proportionality to (a) intensity and (b) time of application of force. Figure 2 illustrates the cardiac response of the monkey to forces up to 6 g given over a period of 50 sec.; the step-like progression of heart rate increase roughly approximates the pattern of g increments.

Increasing the time factor of exposure also affected cardiac responses. Normal monkeys subjected to 6 g for 5, 10, and 20-sec. periods showed progressively greater increases in heart rate, as indicated in figure 3 (73 cases). In the 5-sec. exposure, part of the increase occurred after stopping the centrifuge; at the end of the 20-sec. test, however, the rate fell quickly. Considering the areas covered by the three curves, i.e., below the normal blood level, the comparative values are 1.05 for the 5-sec., 2.00 for the 10-sec., and 4.00 for the 20-sec. test. Under high accelerations, therefore, the overall energy expended by the heart in attempting to maintain or restore normal conditions (e.g., cerebral circulation)

depends considerably on the time of exposure. The data derived from a larger series of animals were somewhat similar (table 1, A).

During long runs of 3 min. or more, the heart rate showed the initial increase, sometimes to twice the normal rate, then gradually fell; usually it became seriously slow at the end of the test (table 1, B). Recovery after stopping the centrifuge was usually rapid. In some cases, if the g forces were not over-severe, the heart rate was maintained at a high level throughout long exposure periods.

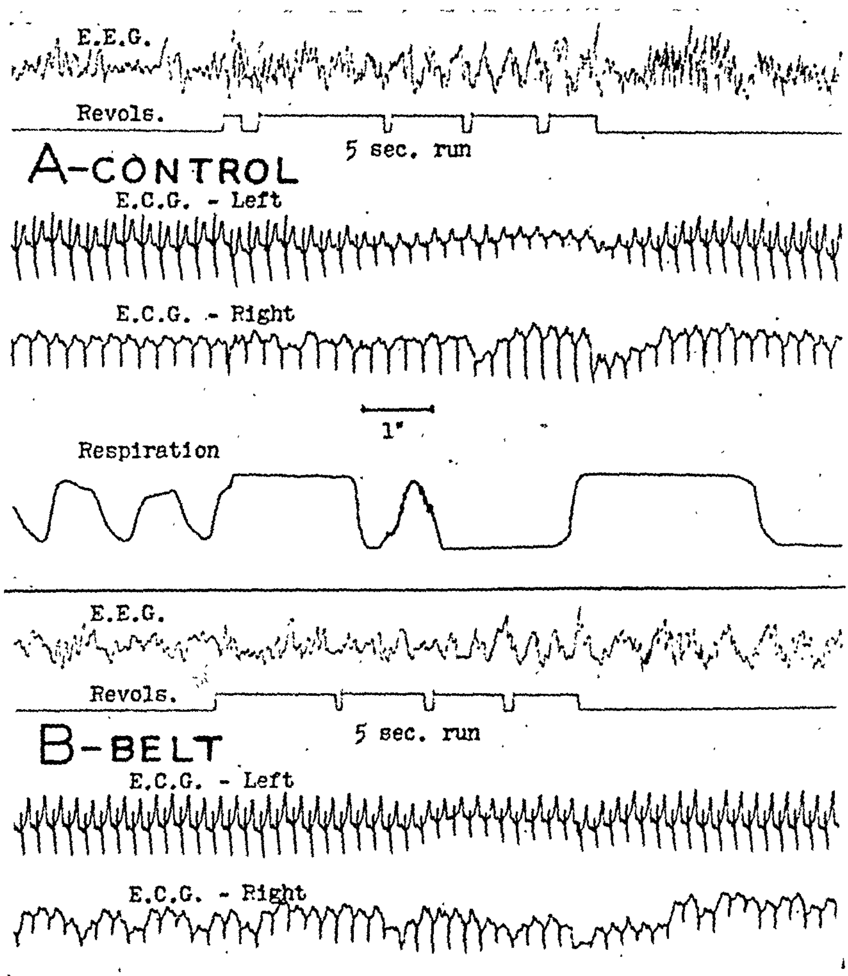

Fig. 1.

Rate and blood-pressure changes during acceleratory exposure are given in figure 4. A frequency-distribution graph showing normal heart rate and the maximal reached in 96 tests at 4 g is also given herewith (fig. 5).

Under negative g forces of brief duration (2–6 g, 5–15 sec.), when the arterial pressure in the head is increased, the heart shows little or no acceleration. In 15 cases (monkey) there was no change, in 16 there was an average decrease of 8, and in 41 tests an increase of 3 beats per min.

E.C.G. Changes in the electrocardiographic record were always observed on

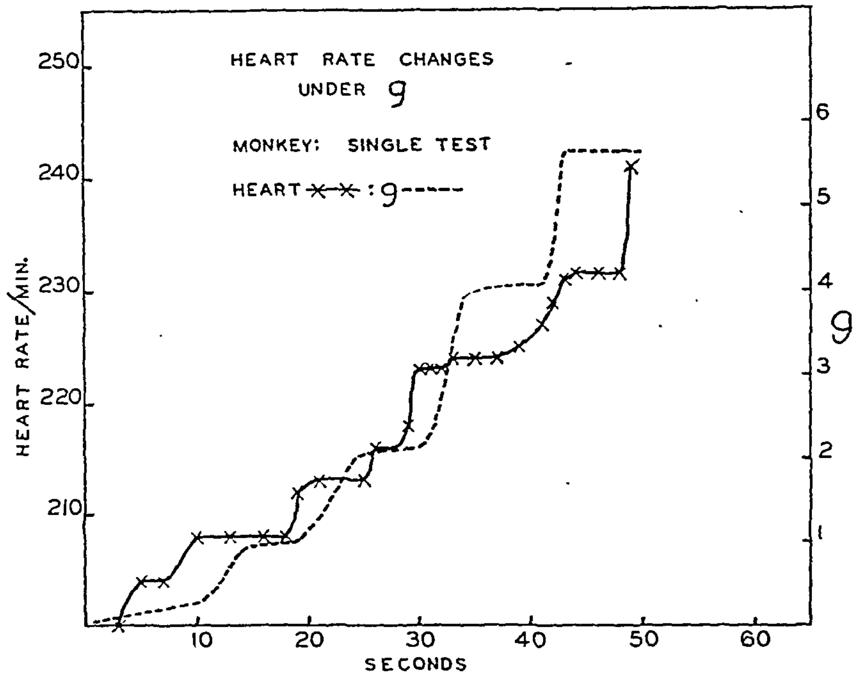

Fig. 2.

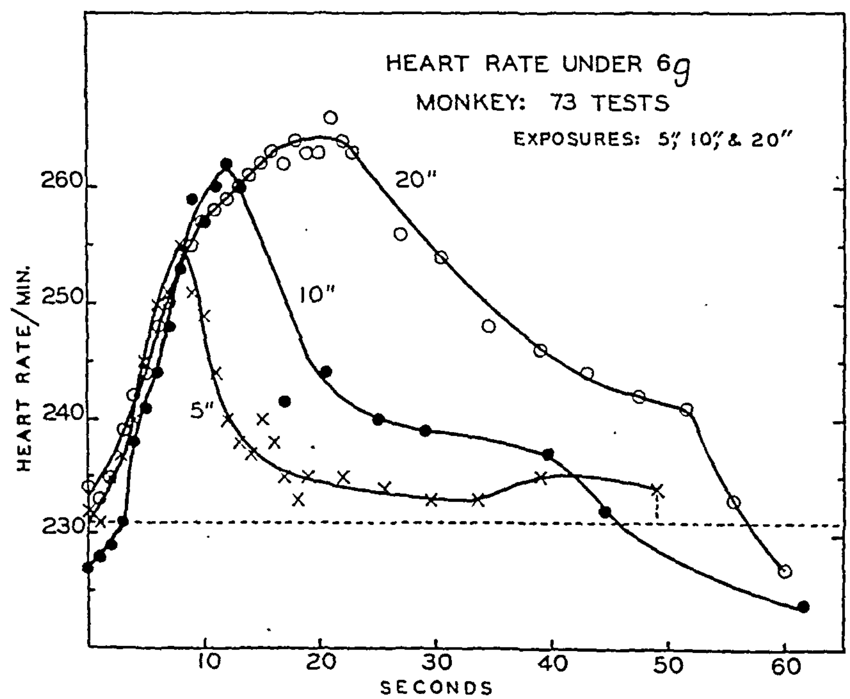

Fig. 3.

centrifugation. Voltages were grossly affected in all leads: in the case of records from Lead I, RS potential fell; in those from Leads II and III, increases occurred.

TABLE 1

Influence of g forces on cardiac activity

A. Monkey: 6 g. Total no. tests 475

DURATION OF TEST	NO. OF TESTS	HEART RATE: PER MIN.					E.C.G.—RS$_4$ AMPLITUDE: MV				
		Before test	*After starting centrif.	End of centrif.	Max. increase	Immed. after centrif.	Before test	*After starting centrif.	End of centrif.	Max. decrease	Immed. after centrif.
sec.											
5	132	220	224	232	12	231	1.76	1.52	1.05	0.71	1.49
10	217	224	229	247	23	249	1.72	1.42	0.75	0.93	1.37
20	84	223	227	248	25	242	1.72	1.39	0.72	1.00	1.25
40	42	211	229	240	29	240					

* Within 3 sec. of start of run.

B. Monkey: E.C.G. changes

MONKEY NO.	TOTAL EXPOSURE TIME	CONDITIONS	Q-T SECS. (1)	R-R′ SECS. (2)	Ratio (1):(2)
	secs.				
A	5	Before test	0.176	0.237	0.744
		Under 6 g—At 4″	0.163	0.232	0.703
		5″ after stop	0.171	0.230	0.744
B	5	Before test	0.192	0.266	0.722
		Under 6 g—At 4″	0.169	0.233	0.726
		5″ after stop	0.205	0.280	0.733
C	10	Before test	0.175	0.248	0.706
		Under 6 g—At 8″	0.165	0.232	0.712
		5″ after stop	0.172	0.240	0.717
D	10	Before test	0.187	0.257	0.728
		Under 6 g—At 8″	0.163	0.237	0.689
		5″ after stop	0.168	0.237	0.709
E	570	Before test	0.185	0.250	0.740
		Under 4 g—At 60″	0.172	0.231	0.745
		Under 4 g—At 125″	0.167	0.231	0.723
		Under 4 g—At 300″	0.173	0.588	0.294
		Under 4 g—At 360″	0.175	0.508	0.345
		Under 4 g—At 420″	0.180	0.510	0.353
		Under 4 g—At 480″	0.165	0.547	0.302
		Under 4 g—At 570″	0.210	0.767	0.274
		After stop At 30″	0.182	0.277	0.658
		After stop At 90″	0.171	0.292	0.586
		After stop At 225″	0.180	0.268	0.672

Two modified leads were preferably adopted for most tests; these were undisturbed by skeletal muscle potential changes developed during occasional movement of an animal. The fifth intercostal space at the apex beat and the contra-

lateral homologous area were utilized proximally, each in circuit with the opposite pinnal root as the remote electrodal area. No differences in results were found, however, when the homolateral ear or various cranial areas were used for the

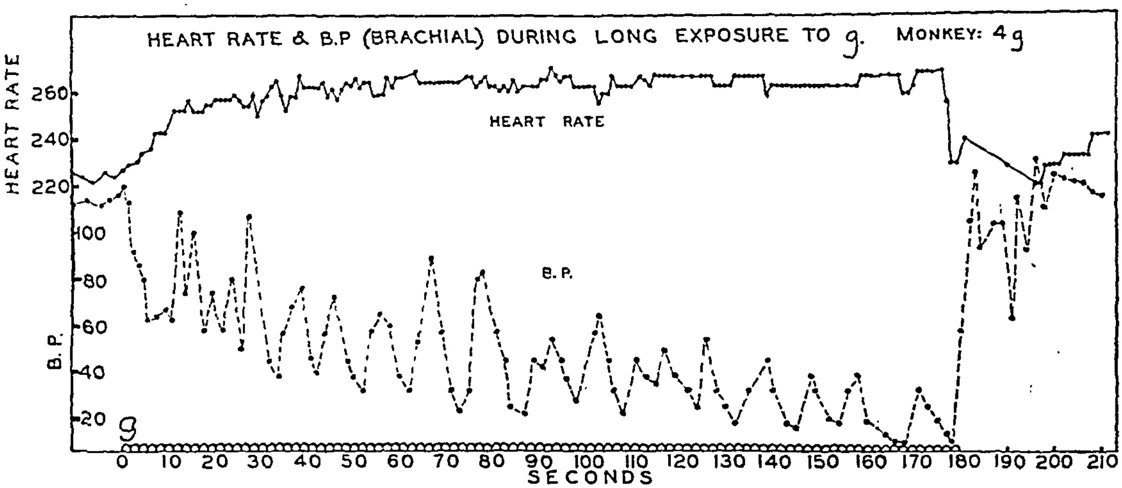

Fig. 4.

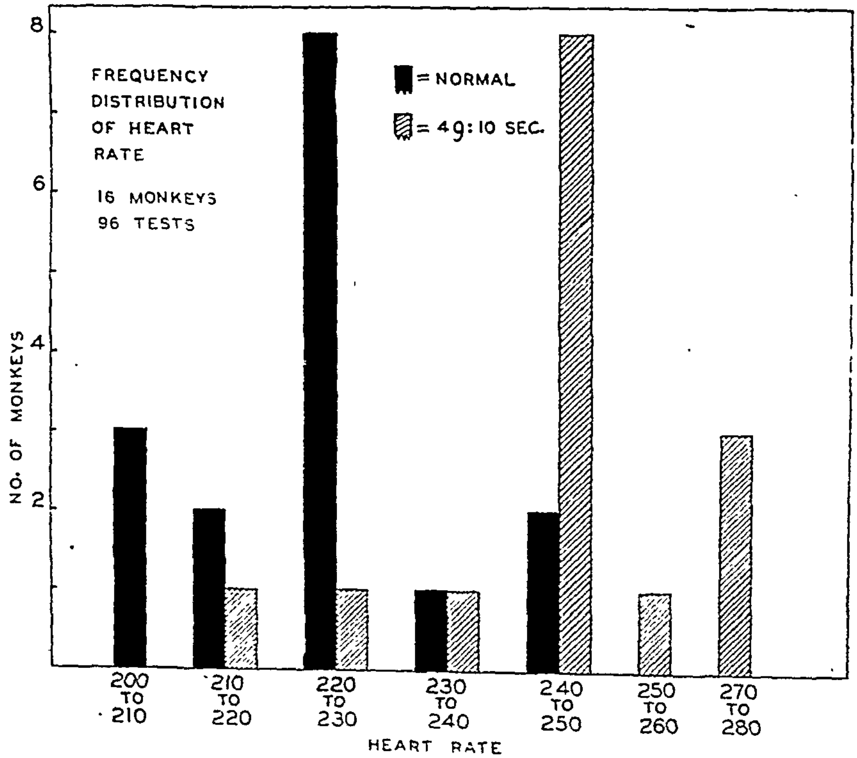

Fig. 5.

indifferent electrode placement. In all routine procedures, therefore, essentially monopolar leads were involved. Careful placement of electrodes was important and gave uniform results.

E.C.G. records obtained from the left precordium (a modified Lead IV) during exposure to high positive g forces showed marked RS (designated RS_4) voltage reduction, while simultaneously from the right chest lead a striking increase in RS potential was observed. Correlatively the T-wave was reduced in the former and increased in the latter case. Records derived from right-left chest electrodes showed moderate RS potential reduction, and striking decrease in the T-wave, during exposure.

Decreases in RS_4 amplitude on centrifugation are shown in table 1, A, and figure 1. A reciprocal correlation between RS_4 change and heart rate during g exposure is observed. Position and slope of the linear relationship varied in different individuals tested.

An analysis of the PQRST complex seen on exposure of monkeys to 6 g for 5 and 10 sec. periods showed expected changes in P-R, Q-T and cycle length consonant with increased heart rate. There was a tendency, however, towards reduction of the Q-T/R-R′ ratio at the end of centrifugation; this became more marked in long exposures. In table 1, B, are shown data derived from a 9.5 min. run at 4 g, i.e., a moderately high acceleration for the monkey. When the heart beat became very slow towards the end of the exposure, the Q-T/R-R′ ratio fell to almost one-third of the normal level; while there was a quick rise within the first few seconds after stopping the centrifuge, establishment of pre-test conditions did not occur until several minutes later. The P-R interval showed a reduction towards the end of the run, but rose sharply in value (lengthened) at the stop, and for a few seconds after centrifugation. Analysis also showed that the electrical axis of the heart varied 10–15° on exposure to 6 g for 10 sec.

To reach a better understanding of the conditions noted above, especially RS_4 changes, experiments were carried out in which (a) the heart position was altered by direct traction, and (b) blood flow through the heart was varied by clamping and opening the large connecting vessels (aorta, vena cava, etc.) at different times. Chest and head leads for E.C.G. were applied over various areas. The results showed that cardio-potential changes indicated by leads from the body surface were produced largely by position of the heart and in part by the amount of blood flowing through the organ.

Animal position and E.C.G. In our routine experiments, tests were made in the supine, outstretched position. Frequency and potential changes in the E.C.G. record have been observed in both the prone and supine positions, in "head in" (+g) and "head out" (− g) positions.

The possible effect of various arm and leg positions was tested. In all cases—knees drawn up, arms down, and combinations of these positions—no striking alleviatory influence on the usual cardiac disturbances was observed. Frequency and voltage changes were not so marked, however, when the drawn-up knees were pressed over the front of the body (abdomen).

Blood pressure. Carotid, brachial and femoral vessels were used in different tests; with the animal in the supine position, pressures in carotid and brachial were found to be similar. In most cases a maximal reduction of about 50 per cent in the brachial arterial pressure level of cats occurred on exposure to 4 g

for 10 sec. (tabe 2, A). In 22 tests the average decrease was 58 from a pre-exposure level of 106 mm. Hg. Usually, arterial pressure began to fall sharply after centrifugation for two seconds, while the g level was rising. This contrasted with a relatively slower rise in pulse rate, usually in 3 or 4 sec., and (usually) a long post-exposure tachycardia. Arterial pressure changes in the dog under various g levels are shown in figure 6.

Curves of arterial pressure and positive acceleration (monkey) showed a rough

TABLE 2

Protection against acceleratory forces by pelvic belt

A. Cat: Brachial arterial pressure; 4 g, 10 sec. Total no. tests, 52

NO. OF TESTS	BELT PRESSURES	ARTERIAL PRESSURE		
		Before test	During test	Change
	mm. Hg	*mm. Hg*	*mm. Hg*	*mm. Hg*
8	0	90	39	−51
6	300	112	67	−45
8	0	116	51	−65
6	300	125	91	−34
6	0	115	58	−57
18	300	128	98	−30

Average arterial pressures:

Belt pressure 0 mm. Hg...................... 22 cases: Change −58 mm. Hg.
Belt pressure 300 mm. Hg...................... 30 cases: Change −34 mm. Hg.

B. Monkey: 6 g. Test on collapse, shown by respiratory and heart failure, and time of suppression of brain waves

MONKEY NO.	WITHOUT BELT		WITH BELT†	
	Survival time to collapse	Time to extinction of brain waves	Survival time to collapse	Time to extinction of brain waves
1	1′ 40″	22″	3′ 02″	2′ 37″
2	1′ 41″	(1′ 41″)*	5′ 52″	(5′ 52″)*
3	2′ 08″	1′ 24″	7′ 03″	6′ 40″
4	1′ 25″	1′ 09″	8′ 10″	7′ 00″
5	6′ 00″	2′ 49″	11′ 49″	4′ 39″
6	4′ 15″	(4′ 15″)*	11′ 08″	(11′ 08″)*
7	4′ 32″	2′ 10″	5′ 23″	4′ 20″
8	2′ 02″	(2′ 02″)*	16′ 46″	(16′ 46″)*
9	2′ 00″	1′ 22″	10′ 00″	(10′ 00″)*
10	1′ 44″	1′ 40″	11′ 45″	(11′ 45″)*
11	1′ 37″	1′ 37″	9′ 18″	9′ 10″
12	2′ 06″	1′ 35″	4′ 51″	3′ 03″
Averages.......	2′ 36″	2′ 05″	8′ 46″	7′ 35″

* No extinction of brain waves.

† Usually 250 mm. Hg recorded pressure.

TABLE 2—*Concluded*

C. Monkey: 6 g, 10 sec. Total no. tests, 139

BELT PRESSURES (0 - Controls)	NO. OF TESTS	HEART RATE			E.C.G.—RS_1 AMPLITUDE		
		Before test	End of centrif.	Increase	Before test	End of centrif.	Decrease
mm. Hg		*per min.*	*per min.*	*per min.*	*mv.*	*mv.*	*mv.*
0	31	232	256	24	1.79	0.59	1.29
75	31	247	255	8	1.54	1.42	0.12
0	9	244	261	17	1.86	0.79	1.07
100	7	259	262	3	1.32	1.04	0.28
0	20	233	253	20	1.85	0.68	1.17
150	19	249	262	13	1.61	1.55	0.06
0	7	218	251	33	1.82	0.64	1.18
200	4	227	238	11	1.71	1.50	0.21
0	7	209	248	39	1.29	0.49	0.80
250	4	238	256	18	1.36	1.31	0.05

Average heart rates:

Belt, 0 mm. Hg (controls)........................ 74 cases: Change, +24/min.
Belt, 75–250 mm. Hg.............................. 65 cases: Change, +10/min.

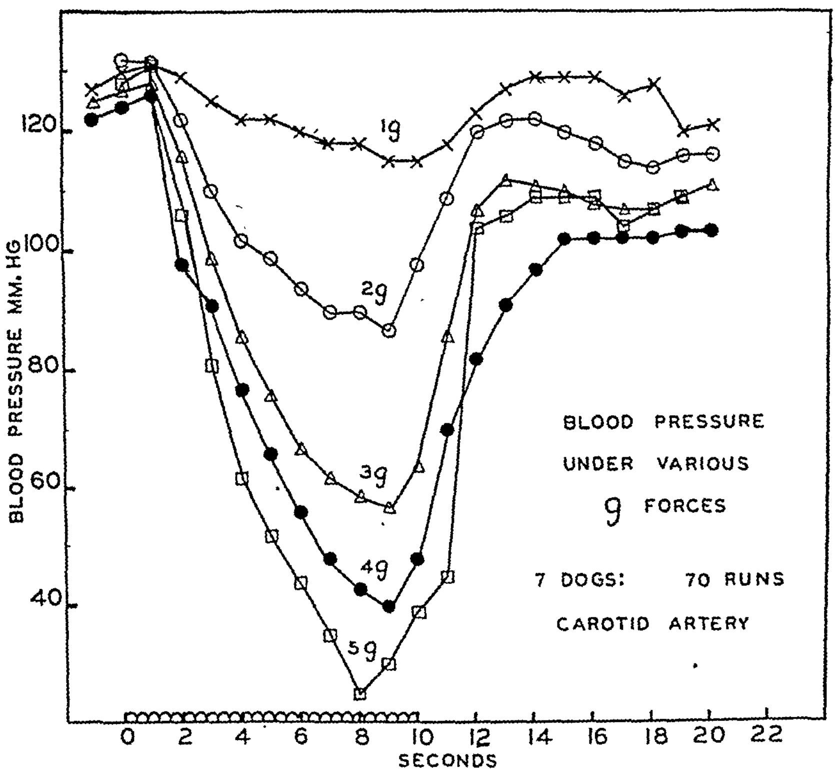

Fig. 6.

reciprocal relationship for the brachial, and a direct relationship for the femoral vessel (fig. 7). There commonly occurred an increase over the resting pressure level within a few seconds after stopping the centrifuge.

Comparative readings of carotid pressure changes under g were taken early in our work with the distal part of the artery (a) closed, and (b) open. After centrifugation, a rebound effect was much more apparent, as expected, in the case of tests with the vessel closed (fig. 8). To preserve conditions as near normal as possible, all experiments following the preliminary tests were carried

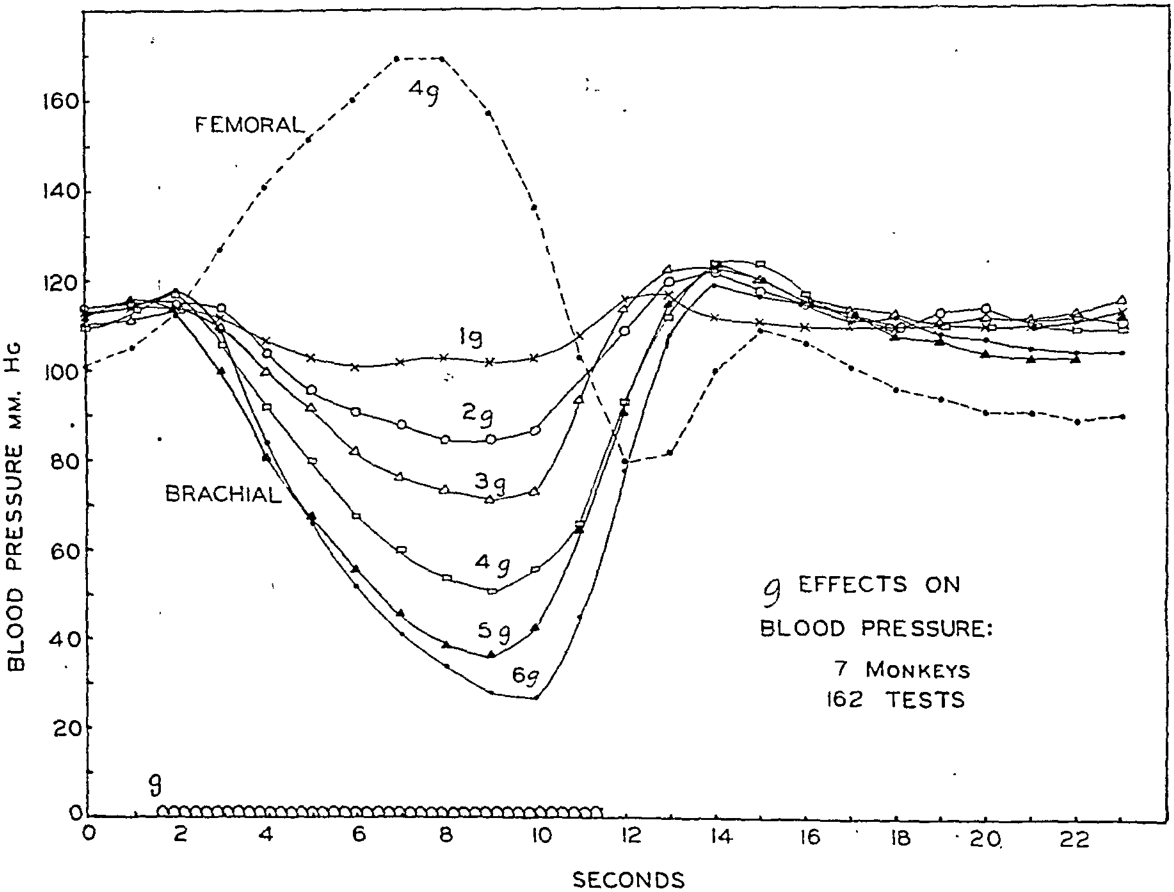

Fig. 7.

out with the vessel open, thus maintaining through circulation in both carotid (or femoral) arteries.

The average minimal or maximal arterial pressures during tests have been plotted as a function of maximal g reached during the runs. For all (positive) exposures a straight line gave a good approximation of the experimental data:

$$\text{Minimal arterial (brachial or carotid) pressure} = 107 - 16 \times g$$
(13 monkeys, 220 tests)

The linear relationship held true for decreases in the brachial as well as increases in the femoral arterial pressures. Thus, a given change in acceleration

produced the same (unit) change in blood pressure at all force levels up to 6 g. In a few cases in which decreases were large per g unit, there were definite indications of reduced resistance on the part of the animal organism.

Using the hydrodynamic model described above in an attempt to imitate the behavior of a living organism, curves of pressure change under g stresses were found to be rather similar to those of brachial arterial pressure. They were,

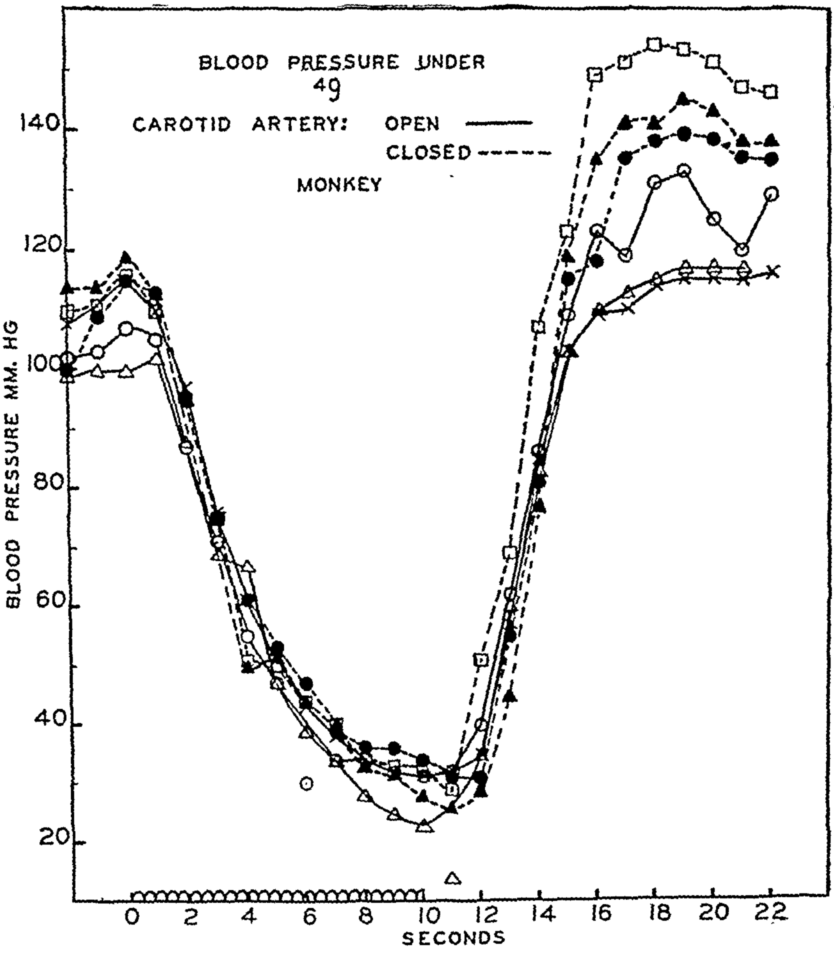

Fig. 8.

however, more U-type than V-type in character (fig. 9). This was probably referable to rigidity of much of the (glass) material of the model, in contrast to the elastic tissue in the animal organism. Also, there were occasional "kinks" in the arterial pressure curves. Considering the minimum water pressures reached in the model under different exposures against maximum g force, the relationship was a straight line for which the equation is:

$$\text{Minimal water pressure} = 116 - 16.5 \times g$$

Blood flow. Reduction of blood flow in the head end of an animal (dog, monkey) subjected to positive g forces could be detected within one or two seconds

after the test was started. A minimal level was reached in a standard 10-sec. run from 4 to 6 seconds after the start of centrifugation. There was a general correspondence with the changes in arterial pressure taken simultaneously, as shown in the curves herewith. At 3 or sometimes 4 g the flow in the carotid artery usually reached zero levels, or even showed slightly negative values. The depressions in blood flow showed a proportionality to applied g forces. After stopping the centrifuge a rebound effect, persisting for 30 sec. or more and with flow values reaching 25 per cent above normal, was commonly observed (fig. 10).

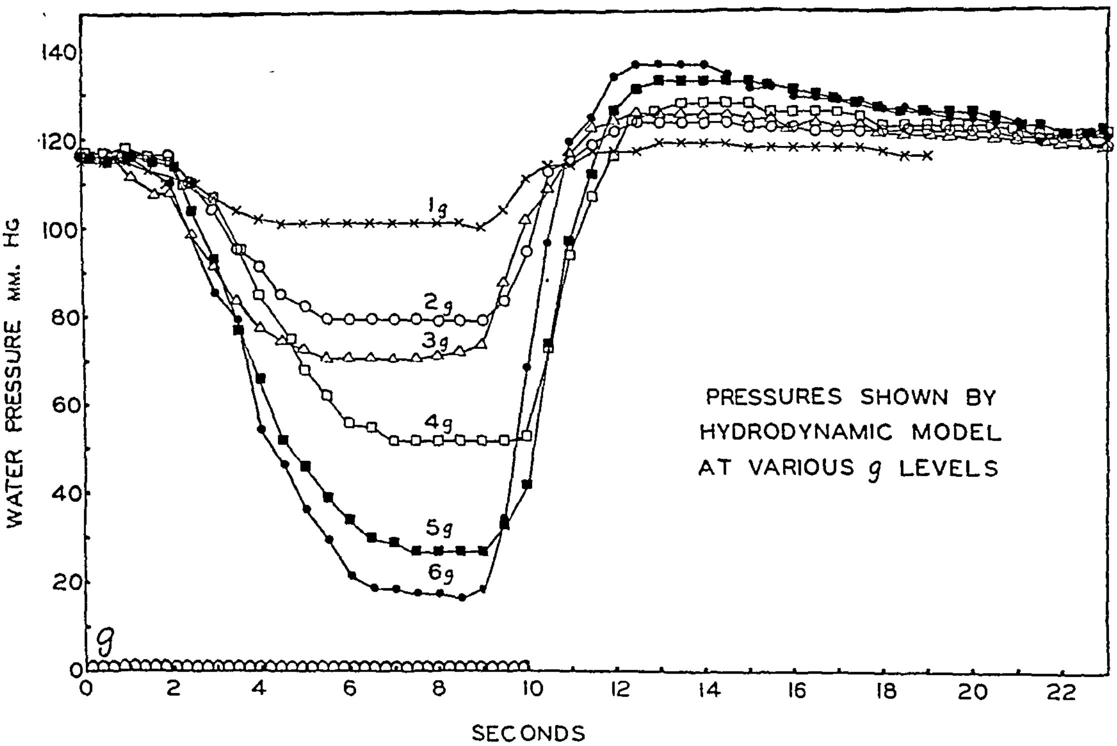

Fig. 9.

The deficiency in blood flow during an exposure, i.e., the cubic centimeters of blood which failed to reach the head end of an animal during a run, was determined by the area below the normal blood flow level. Calculations made from data derived from a large number of experiments fit the equation:

$$\text{cc. deficiency} - 0.82 = 1.7 \ln g$$

This relationship may indicate that blood flow changes are not a function of g difference as such, but of $\frac{\Delta g}{g}$ a phenomenon observed in certain physiological activities.

Transverse g effects. Several dogs and cats were given 10 sec. exposures to forces up to 8 g applied in the transverse direction, left to right, with the animal

in the supine position. No significant effect of forces up to 3 g was noted; under 4–8 g, however, carotid blood pressure and flow increases from 10–15 per cent above normal were often found, with accompanying increases in heart rate.

Respiration. In all animals tested (monkeys, dogs, cats, rats), respiratory rate was inhibited throughout most of the acceleratory period; only occasionally were brief increases in rate noted. Often the depth (volume) of respiration was increased for short periods. In 256 tests on monkeys the respiratory rate showed an average decrease of slightly more than 50 per cent during tests at 6 g

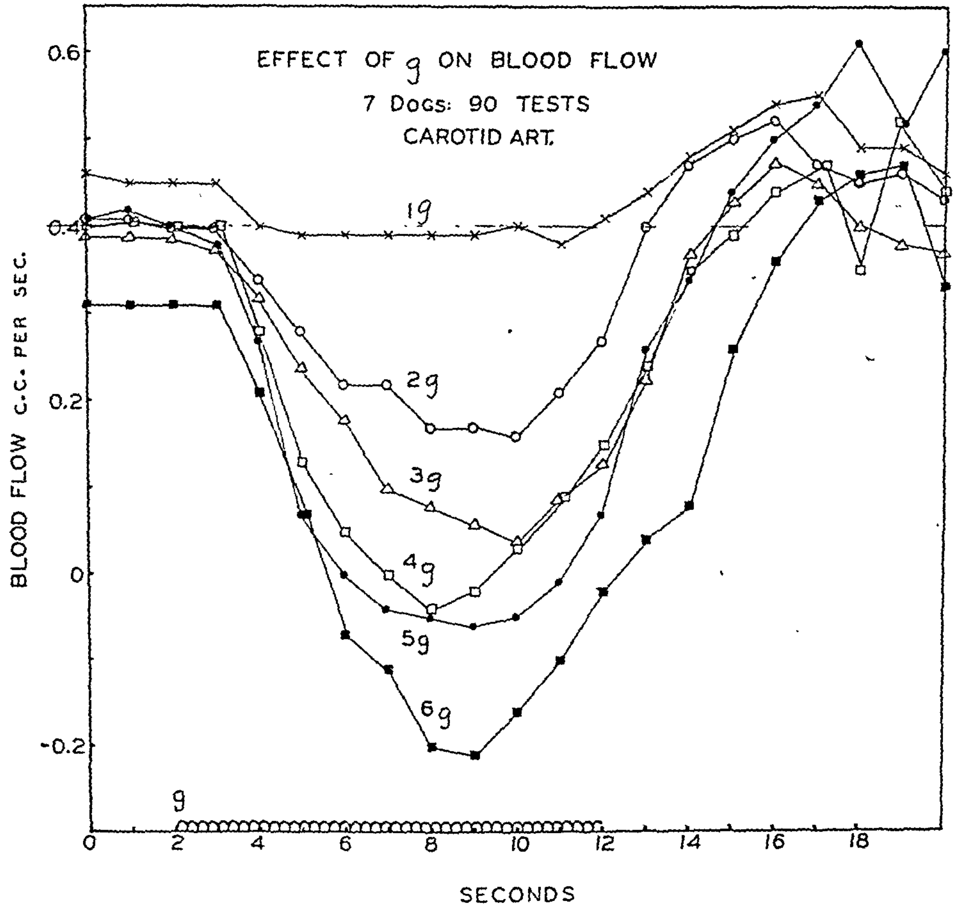

Fig. 10.

for periods ranging from 10 sec. to 6 min. After exposure the rate increased between 5 and 10 per cent for a minute or so.

E.E.G. In most experiments monkeys were used, and records were taken continuously throughout the tests. "Delta" brain waves, or those of high voltage and low frequency, commonly appeared quickly (within the first second) on exposure to high acceleratory forces. They usually continued throughout 5-sec. tests, but sometimes disappeared towards the end of 10-sec. exposures; after stopping the centrifuge, deltas persisted, or reappeared and lasted a further 5 to 15 sec. On repeated exposures being given, the brain waves commonly showed larger amplitude and longer persistence.

The incidence of deltas was greatest during the longer runs of 30 sec. or more (fig. 11); during this period they showed a probability of occurrence of almost 1.0 in tests at 6 g. In comparison, the probability figure was 0.73 during 10-sec. tests. For post-exposure deltas, the probability was about 0.7 for 20-sec. or longer runs.

While delta waves were characteristically seen, the effect of acceleration on medium-frequency, medium-voltage waves was less noticeable. The only change in the latter was a slight rise in frequency whenever a change in g forces occurred, i.e., there was a small rise on beginning centrifugation and also when the centrifuge stopped.

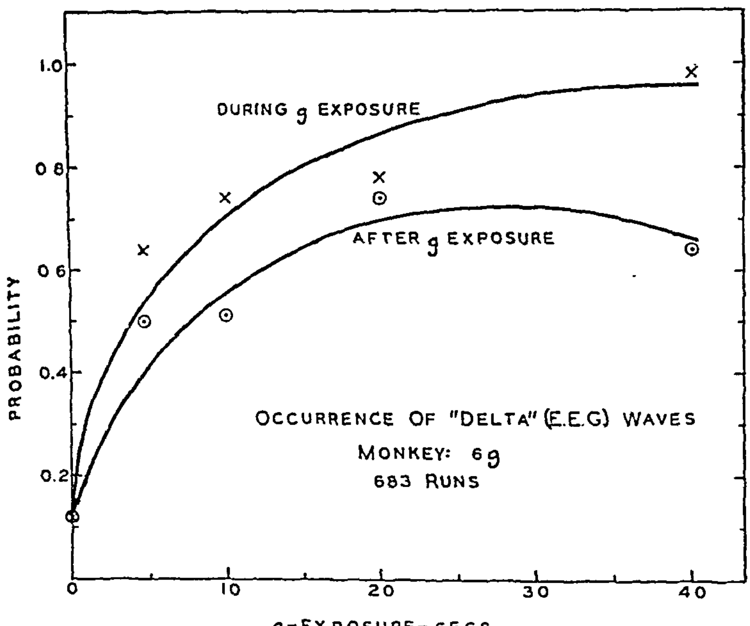

Fig. 11.

"Alpha" waves were commonly present in the resting monkey (eyes bandaged); their incidence was quite variable—from 5–60 per cent of the record examined. The average frequency was about 7.7 per sec., and the average duration of bursts about 1.4 sec., in 240 determinations. These waves were abolished by centrifugation.

"Experimental neurosis" and other tests. Attempts were made to produce "experimental neuroses" by frequent exposure of monkeys to high g forces. Animals were given runs on the centrifuge two or three times weekly. About 50 runs at 5 g were made on each of 6 monkeys during an experimental period of several weeks. No treatment was given in 3 cases; in 3 others benzedrine (for "sensitization") was given before every exposure. A siren was sounded close to the animal for 10 sec. immediately before each centrifuge run, and later the possible effects of the siren alone were tested.

In no case, after two months of such tests, were any significant detrimental (cerebral) effects noted. There were moreover only slight signs of conditioned

reflex development (by cardiac changes preceding a run) in a few instances. Dogs tested daily on the centrifuge at various g levels over a period of 6 months also showed no emotional instability; indeed, some animals appeared to enjoy the experience.

Protective factors: Abdominal belt. The use of a pelvic or abdominal belt under pressure (usually a sphygmomanometric cuff, inflated to 150–300 mm. Hg) by an animal undergoing exposure to g forces gave protection to many of the body's functions. Survival time to collapse was found to be greatly prolonged, and time to produce extinction of brain waves was markedly lengthened. In most of these cases the time element was multiplied many times (table 2, B).

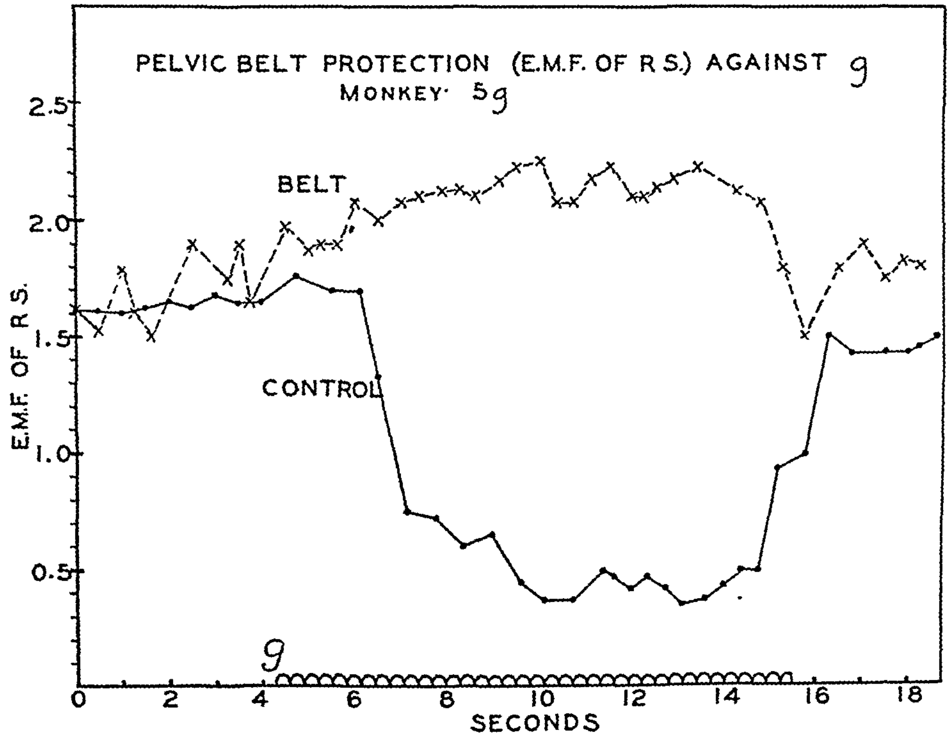

Fig. 12.

As shown by the electrocardiographic record, RS_4 amplitude was maintained with only slight decrease under 6 g in the case of belted monkeys, compared to severe reductions in the same animals when unprotected. Tachycardia was also much less under g when abdominal pressure was applied (table 2, C; figs. 12 and 13).

Animals also showed maintenance of arterial pressure nearer normal levels when given abdominal belt protection during acceleratory exposures. The advantage varied between 1 and 3 g, inversely to the severity of the exposure (table 2, A). Blood flow reductions under moderate g forces were about half as great only, when an abdominal belt was applied. Tests made with an inflatable neck cuff at various pressures gave slight or indefinite results.

Adrenalin effects. A number of tests were made on monkeys and cats, using different doses of adrenalin in oil and in aqueous solution, and various g levels and exposure periods. On giving adrenalin intravenously and exposing the animal to acceleratory forces at intervals thereafter, some protection was afforded during the very short period when arterial pressure was normally elevated, i.e., by adrenalin. The brachial arterial pressure of treated animals was maintained at about normal under 1–3 g forces applied for 5–15 sec.; in longer runs of a minute or two, or under 3–6 g, acceleratory forces overcame the hypertensive action of adrenalin. Even large doses given in oil offset for a short while only the influence of high accelerations.

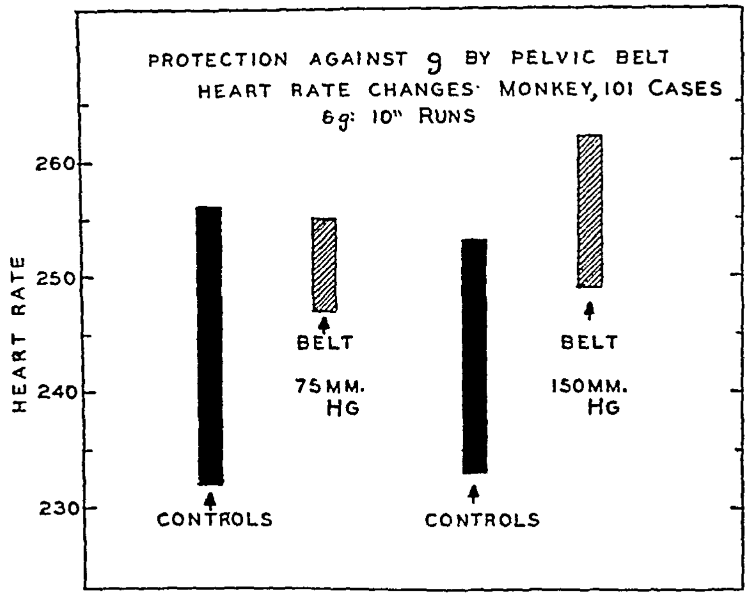

Fig. 13.

Discussion. Decrease of arterial pressure in the head end of the animal was specifically correlated with increase in heart rate during exposure to positive g. Femoral pressure showed at the same time a marked rise. When an abdominal belt or other protective measures were used, the (usual) heart rate and carotid pressure changes under g were reduced correspondingly.

Little or no heart rate increase occurred on exposure of animals to g forces while under low barometric pressures, although the carotid pressure level was sharply decreased. In this case suppression of the tachycardic response to g forces was probably referable to the severe decrease in oxygen supply to the heart tissues.

During negative g tests, when carotid pressure was increased, the heart rate of dogs showed insignificant change. A paradoxical bradycardic reaction was noted, moreover, towards the end of prolonged exposure to positive g, when the central nervous system was in serious need of blood supply. This probably represented a failing myocardium, in view of the attendant severe oxygen lack. Rapid recovery of normal cardiac activity appeared on stopping the centrifuge.

Results indicate clearly that the so-called "Marey Law" (Marey, 1863) is operable normally and primarily in the event of reduced circulation to the cerebral tissues. Not even in this case does the "law" hold if moderate anoxia is induced, and cardiac acceleration may fail to occur although the brain is in great need of vascular support.

Sharp changes in E.C.G. during centrifugation, although explained on the basis of heart and blood shifts, afforded striking criteria of the effects of g forces on the body. The protection afforded by an abdominal belt was clearly shown by preservation of normal E.C.G. voltages.

Curves of blood pressure (and blood flow) derived from animals given 5 or 10 sec. runs on the centrifuge at various g levels were not usually smooth in character. There was thus some indication of the initiation of reflex activity, apparently in an attempt to offset cerebral ischemia, during acceleration. Slight irregularity of pressure and flow curves, suggestive of carotid sinus intervention, appeared within 6 or 8 sec. after the start of centrifugation.

In longer runs gross waves of arterial pressure change persisted for a minute or two under severe g forces; they continued for some time after a run, indeed, when the carotid pressure had reached normal or supernormal levels. The response of pressure-regulating mechanisms to gravity, somewhat slow in starting, may therefore be projected in time long after the initiating stimulus has been withdrawn.

Often a hundred or more tests were made, it may be noted, to establish definitely curves of change (of blood pressure, blood flow, etc.) produced during exposure of a series of animals on the centrifuge. Spread of points derived from an individual or group was nevertheless not considerable, in view of the rather severe experimental conditions involved.

The incidence of "delta" brain waves during and for sometime after centrifugation was probably referable to the induced cerebral anoxia. Led by this idea, oxygen administration was tested, without producing any significant benefit. The use of CO_2, however, was found to be advantageous in raising resistance to acceleration. On giving CO_2 (usually but not always with O_2) immediately before exposure, it appears likely that cerebral blood flow was augmented; this constituted a preparatory, resistant factor, and conferred a 1–2 g advantage on the animal.

The protection provided against g forces by an abdominal cuff under pressure was strikingly shown by a number of different criteria used. In 4 cases, monkeys given such protection withstood 6 g for periods of 10 min. or more, compared to the control average of 2.5 min. shown by the same animals (table 2, B). One protected animal survived a force of 6 times gravity for nearly 17 min., without showing cerebral (E.E.G.) failure.

Little indication can be given now of the work done on acceleration by many groups during the war. Reference may be made, however, to the splendid accomplishments of the Mayo aero-medical unit, a partial report of whose work has recently appeared (Wood *et al.*, 1946). Other workers have also published abstracts of their experiments in the past two years (Clark, Drury; Maaske *et al.*; 1945–1946).

In a day when not only interprandial ocean-spanning is projected, but subterranean (vacuum-tube) as well as stratospheric travel at supersonic speeds by atomic propulsion is considered by high authorities, functional studies of those unique reactions provoked by high acceleratory forces must surely be given greater emphasis. That aircraft performance easily outranks the resistance of the human mechanism is and will always be true. Biological investigator and engineer must rub wits together quite often, however, if the gap between mechanical and biological capacities is to be kept within reasonable (or survival) bounds. A timely, thorough and provocative consideration on this subject by McFarland (1946) should be committed to mind and heart by physiologists and all who control aircraft output of any sort.

Through vigorous prodding by war and its arbitraments, much fruitful work in this and near-related fields resulted. Now, simply to enjoy the argosies of peace (not to urge any idea of preparedness), it should at least be possible to maintain medico-physiological inquiry on a level commensurate with that of technological progress. Meanwhile findings such as those herein on one phase of the problem may simply be added to others more forcibly impressed by increasing contingencies in this sphere of man's activity.

SUMMARY

A 20-foot diameter heavy steel centrifuge with blood-pressure and other devices attached for determining effects of accelerations up to 50 g is described. Results of tests on a large number of monkeys, dogs and cats are given.

Acceleration of the heart rate (5–30 beats) on exposure to positive g forces shows a proportionality to the intensity and time of application of the force. Under prolonged exposure (5–10 min., 3–4 g) bradycardia and irregularity set in, but recovery was rapid on stopping the centrifuge. Negative g forces produced no significant change in heart rate (dog).

E.C.G. records derived from the left precordium showed marked RS_4 voltage reduction during positive g exposure, while increase in voltage was shown by the right chest record obtained simultaneously. Experiments indicated heart position and blood content were the responsible factors.

Carotid (or brachial) blood pressure fell sharply under positive g forces, and the femoral pressure rise was even more abrupt. Arterial pressure changes (carotid, brachial, femoral) showed a linear relationship to applied g stresses. A given change in acceleration produced the same unit change in arterial pressure at all force levels up to 6 g.

The carotid pressure of monkeys under g forces was affected significantly less than that of cats and dogs. At forces between 1–6 g, monkeys showed about 1 g advantage over the latter animal types.

Changes in pressure in a hydrodynamic model under acceleratory forces were similar to arterial pressure changes found in animals.

A carotid sinus reflex response affecting arterial pressure appears to be initiated 6–8 sec. after starting exposure to high g forces.

Blood flow reductions in the carotid artery occurred within a second or so after starting the centrifuge, and showed a proportionality to the acceleration

applied. Under a force of 3 or 4 g for 10 sec., flow in the carotid or brachial vessel usually reached the zero level.

Transverse forces (across the body) of 4–8 g produced moderate increases in heart rate and carotid pressure and flow.

On starting centrifugation, high-voltage low-frequency ("delta") brain waves appeared in 75 per cent of monkeys tested within a second or so; sometimes they disappeared towards the end of a 10-sec. run, and appeared again when the centrifuge stopped. The probability of occurrence of "deltas" was almost 1.0 on exposure to 6 g for 30 sec.

Attempts to produce conditioned reflex responses and "experimental neuroses" by exposure to high g forces applied frequently over several months were negative.

Considerable protection against g forces was afforded by a belt or cuff around the abdominal area inflated usually up to 300 mm. Hg pressure. Survival time, E.C.G. and E.E.G. values, and arterial pressure and flow levels were all significantly affected. The advantage afforded by abdominal belt pressure varied between 1 and 3 g, inversely to the severity of the exposure. Adrenalin gave moderate and very brief protection.

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THE DISTRIBUTION OF TOTAL ELECTROLYTE, POTASSIUM AND SODIUM IN THE CEREBRAL CORTEX IN RELATION TO EXPERIMENTAL CONVULSIONS[1]

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The results of many physiochemical studies of the disordered physiologic processes of the brain in convulsions (1) have led McQuarrie (2) to state that "the characteristic abnormality in epilepsy involves a disturbance in the surface functions of the brain cell membranes." This conclusion is strongly supported by the work of Spiegel and Spiegel-Adolf (3–5) and of Spiegel, Spiegel-Adolf and Henny (6, 7). They demonstrated by means of an electrical method for measurement of cerebral cortical polarization that cortical permeability (which has an inverse relationship to polarization) is increased by various experimental conditions which produce a lowered convulsive threshold or actual convulsions

Cicardo and Torino (8, 9) also concluded, on the basis of their recent extensive work, that all bioelectric potential changes, including those associated with the disordered nervous discharges of convulsions, are the result of shifts of electrolytes which are associated with changes of permeability of cellular membrane. They reported that cerebral venous blood plasma of the dog contains an increased amount of potassium during induced convulsions, and that this additional potassium represents a loss from the brain since such increases occurred only in cerebral, and not in systemic, venous blood. Similar work by Kendall and Essex (10), however, is not in agreement with Cicardo and Torino's findings, for they were unable consistently to detect a significant increase of potassium content of cerebral blood resulting from experimental convulsions.

Liebert and Heilbrunn (11) have used the method of micro-incineration to study the effect in vivo of metrazol and insulin on the mineral content of the brain of the guinea pig and of the rabbit. They reported that these substances induced changes of total ash in the cornua Ammonis and in various cortical areas. Micro-incineration in itself does not provide qualitative information on the various ash components, however, and in the present study a microcrystallographic method was employed for analysis of micro-incinerated preparations, to determine what changes of local distribution of potassium and sodium in the cerebral cortex might result from experimentally induced convulsions.

METHODS. Each experiment consisted, essentially, in taking a small control specimen of cerebral cortex from an animal, after which the animal or a litter mate was given one or more convulsions. Then a second cortical specimen was

[1] Abridgment of portion of thesis submitted by Doctor Colfer to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Ph.D. in Medicine.

removed for comparison with the control. The distribution of electrolytes was studied by means of micro-incineration and crystallographic analysis of the ash of the micro-incinerated specimens.

The experimental animals used were rats and rabbits. In all cases specimens were taken from the cerebrum in the form of small disks of a standard size: 6 mm. in diameter by 2 mm. thick. A sharp cork borer served to make clean circular cuts perpendicularly from the cortical surface into the tissue, and a very small scalpel, introduced as nearly parallel as possible to the cortical surface, was used to complete the separation of the disk. In most of the experiments a control specimen was taken from one side of the frontal cortex through a trephine in the skull and the second specimen was taken, after the animal had been placed under the desired experimental conditions, through another trephine, from the corresponding area of the opposite hemisphere. The animal was anesthetized with ether, or by local infiltration of the scalp with procaine, while the two trephines were prepared. In other experiments a separate animal was used to supply control specimens. The control animal and the one on which the experimental procedure was done were killed by bleeding from the carotid arteries following etherization. In these cases craniotomy was then quickly done, and the brain was thus obtained for removal of the disks from more diversified areas than was possible in the first type of experiment.

In the majority of experiments convulsions were induced by the passage of an electric discharge through the animal's head, following a technic similar to that used in electroshock therapy of psychiatric patients (12). In other cases metrazol was injected intravenously to produce the convulsions, and in many of the experiments with rats convulsions were brought about by exposing the animals to a loudly hissing air blast (audiogenic convulsions) (13).

The electroshock apparatus consisted of a variable transformer circuit operating from a 110 volt, 60 cycle, line current. The circuit was controlled by an x-ray timer, and the output potential amounted to 250 volts. The current output could be regulated quite exactly, by adjustment of the transformer, through a range of zero to 135 milliamperes. The electrodes used consisted of small coils of braided copper wire which were applied bilaterally to the animal's shaved temporal scalp by means of adhesive tape. Electrolytic jelly was employed to provide good contact and reduce resistance.

For micro-incineration of the cortical specimens a micro-incinerator of Scott's design (14), and his technic (14), with slight modification, were used throughout. The tissue fixative employed was absolute alcohol alone, rather than a mixture of formalin and alcohol. It was found that the former brought about satisfactory fixation, whereas the 60 per cent water content of concentrated formalin was theoretically objectionable since it was important that the normal distribution of water-soluble tissue salts be preserved. All sections were cut at 12 microns.

Analysis of the micro-incinerated tissues for sodium and potassium was accomplished by a micro-crystallographic method (15). This involved either of two procedures: 1, addition under the microscope of measured microdrops of reagent directly to selected areas of the micro-incinerated specimen and observa-

tion of the crystals formed as a result of reaction between the ash and the reagent, or 2, taking into solution under the microscope particles of ash from selected areas of a micro-incinerated specimen, and subsequently analyzing this solution by means of crystallographic tests carried out in capillary tubes. These operations involved the use of a micromanipulator which would make possible the accurate placement of measured microdrops from a capillary pipet on selected microscopic areas of the micro-incinerated preparation, and which would also allow for collection into another capillary pipet of microdrops so placed on the specimen. A moist chamber was necessary to prevent evaporation of the microdrops (15). Rough estimation of the amount of sodium or potassium in the ash tested, as compared with that in similar areas of a control preparation, rested on comparison with the control analyses with respect to the number of crystals obtained (16), their size and the rapidity of their formation (16). The reagents employed were zinc uranyl acetate for sodium and chloroplatinic acid for potassium (17).

EXPERIMENTS AND RESULTS. Experiments were carried out to determine what effects induced convulsions might have on distribution of total electrolyte, and of sodium and potassium, in the cerebral cortex of rats and rabbits. Additional experiments were performed to control the possible influence on the results of such factors as anoxia, muscular activity, anesthesia and cortical topography.

a. Metrazol convulsions. Eight adult rabbits were used. In each case ether anesthesia was administered during preparation of a trephine opening over the right or left frontal lobe, the control specimen was removed and the incision closed. Another trephine was then made on the opposite side of the skull at a position exactly corresponding to the first, the animal was allowed to recover from the anesthesia, and metrazol was given intravenously in a dosage of 0.1 cc. per kilogram of body weight. A cortical specimen was removed through the second trephine after a generalized convulsion had been in progress for thirty to sixty seconds.

The specimens were prepared according to the routine technic, control and postconvulsive tissues from each rabbit being handled together throughout fixing, clearing, infiltrating, blocking, cutting, mounting and incinerating. Each completed slide, as in all the experiments, therefore carried micro-incineration ash of both a postconvulsive and a control specimen.

Examination of the completed preparations under the darkfield microscope at magnification of 900× and 1,200× showed definite differences between the control and postconvulsive specimens. The latter showed a heavy, diffuse, intraneuronal ash which lacked nuclear markings, while in the controls there were intraneuronal concentrations of ash in a position corresponding to that of the nuclei, with relatively less cytoplasmic ash. The intercellular ash of these preparations has been difficult to estimate, because of the prominence of the ash patterns of the neurone processes. In the superficial, granular layer of the cerebral cortex, however, where there are relatively fewer neurone processes, the intercellular ash could be better estimated. It showed no significant change of concentration resulting from induced convulsions. Neuroglia cells have not shown

a prominent ash content in any of these experiments, nor have any changes of neuroglial ash been found to result from convulsions.

The micro-incinerated preparations were now analyzed for neuronal sodium and potassium by the crystallographic methods described earlier in this paper. Numerous analyses of the preparations of each rabbit were carried out by both the "on-slide" and the "capillary tube" method. These analyses consistently indicated that a neuronal loss of potassium and a gain of sodium were associated with metrazol convulsions (figs. 2 and 3). This information on the sodium and potassium of *neuronal* ash were obtained by selecting for analysis only those microscopic areas where there were considerably more ash patterns of neuronal cell bodies than in the average microscopic field of the preparation.

TABLE 1

Experimental conditions and results in a series of animals given electroshock

RABBIT	ELECTROSHOCK CURRENT, MILLIAMPERES	ELECTROSHOCK DURATION, SECONDS*	NUMBER OF ELECTROSHOCKS AND CONVULSIONS	RESULTS: POSTCONVULSIVE NEURONAL ELECTROLYTES COMPARED WITH CONTROL
1	100	0.2	4	Diffuse increase of total intraneuronal ash. Diminished potassium, increased sodium
2	90	0.2	6	Same as (1)
3	135	0.2	10	Same as (1)
4	135	0.4	10	Same as (1)
5	80	0.2	2	Questionable increase of total ash and no demonstrable diminution of potassium or increase of sodium
6	80	0.4	1	Same as (5)
7	135	0.2	1	Same as (1)
8	135	0.2	4	Same as (1)
9	135	0.1	2	Same as (1)
10	135	0.1	2	Same as (1)
11	135	0.2	4	Same as (1) except no demonstrable change of sodium
12	135	0.2	4	Same as (1)

* The duration of convulsions produced by electroshock ranged from 10 to 40 seconds.

b. Electroshock convulsions. Twelve adult rabbits were studied to observe the effect of electrically induced convulsions on the distribution of total ash, sodium and potassium of the cerebral cortex. Table 1 indicates the experimental conditions and results for each case.

Removal of specimens from the cerebral cortex of control and postconvulsive animals was done in the same manner as in the preceding series and, similarly, ether anesthesia was used during trephining and removal of control specimens except in the cases of rabbits 5, 6, 9 and 10, in which local infiltration anesthesia was used. The intervals between induction of successive convulsions in each case were one to five minutes. Postconvulsive specimens were removed during the course of the last convulsion, or within a few seconds after it had ceased,

except in the cases of rabbits 11 and 12, in which an interval of thirty minutes was allowed.

The changes of distribution of total ash (fig. 1) were similar to those which occurred with metrazol convulsions. Changes of intraneuronal concentration of sodium (fig. 2) and potassium (fig. 3) were also similar to those of the preceding series, though they were not quite so constant, since they were not detected in the cases of rabbits 5 and 6 in which a relatively low electroshock current was used.

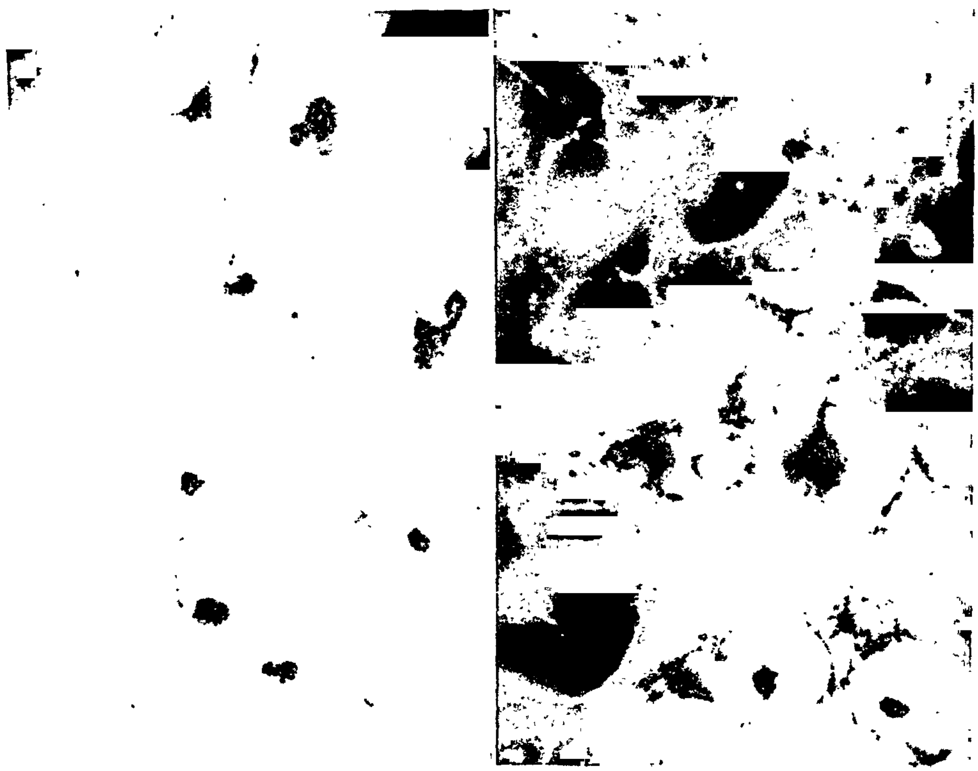

Fig. 1. Effect of experimental convulsions on the total electrolyte of the cerebral cortex of the rabbit. *Left:* Micro-incinerated control preparation (×900). The ash of neuronal cell-bodies appears as concentrations of light-refractive material. *Right:* Micro-incinerated preparation of the corresponding cortical area, opposite side, of the same animal after electroshock convulsions (×900).

In the cases of rabbits 8 to 12, inclusive, an additional cortical specimen was removed after three to three and a half hours. Micro-incineration and crystallographic analysis of these specimens showed no significant difference from the control specimens, and therefore it was concluded that the described effects were reversible within three and a half hours.

c. *Audiogenic convulsions.* Eight adult white rats, found on testing two weeks previously to be "audiogenic reactors (13)," and eight control animals, each of which was a litter mate of a corresponding experimental animal, were studied for the effect of this type of convulsion on the electrolyte content of the cerebral cortex.

Each of the audiogenic reactors was subjected to the sound stimulus (a loudly hissing air jet) and each had a typical, generalized convulsion. In every case

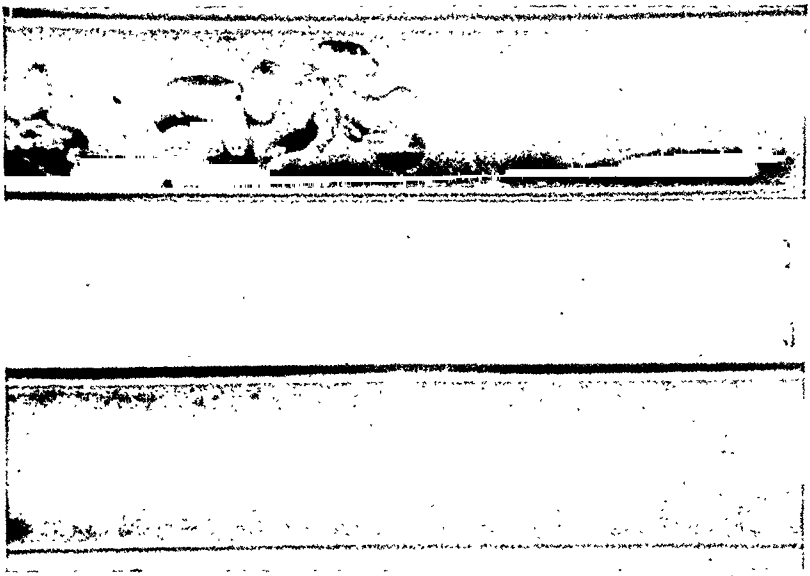

Fig. 2. Results of microcrystallographic analysis for sodium of micro-incinerated preparations shown in figure 1. *Above:* Sodium zinc uranyl acetate crystals obtained in capillary tube from ash of neuronal areas of the postconvulsive preparation (×200). *Below:* The relative absence of crystals obtained on similar analysis of the control preparation (×200).

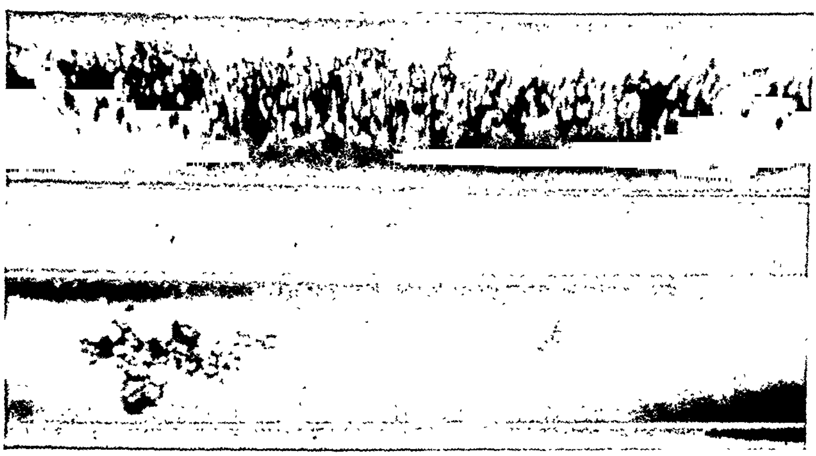

Fig. 3. Results of microcrystallographic analysis for potassium of micro-incinerated preparations shown in figure 1. *Above:* Potassium chloroplatinate crystals obtained in capillary tube from ash of neuronal areas of the control preparation (×200). *Below:* The relative dearth of crystals obtained on similar analysis of the postconvulsive preparation (×200).

the animal was etherized, craniotomy was performed and specimens of frontal cortex were removed and placed in fixative within one to two minutes after the convulsion. Similar specimens were obtained in the same way from the

control rats, though these animals had not, of course, been exposed to the air blast.

Microscopic examination of the micro-incinerated preparations of this material revealed effects of audiogenic convulsions which were of the same nature and extent as those described in the preceding experiments. On crystallographic analysis for sodium and potassium, however, a change was not found so consistently, since only a half of numerous determinations indicated a neuronal loss of potassium and gain of sodium. The remaining determinations showed no change.

d. Asphyxia control. Since severe or mild anoxia accompanies convulsions, it seemed important to determine whether anoxia alone might cause shifts of the electrolytes of the brain similar to those produced by generalized convulsions. Accordingly, three rabbits were used to determine the effect of anoxia alone on the electrolytes of the cerebral cortex.

Each of the experiments was done as follows: A control specimen was removed from the right frontal cortex through a trephine opening made following local infiltration anesthesia. With the animal under local anesthesia, asphyxia was produced and after three minutes specimens were removed from the left frontal cortex.

Comparison of the preanoxic and postanoxic specimens following micro-incineration showed no difference between their ash pattern or neuronal potassium or sodium.

e. Ether controls. Specimens of tissue were frequently obtained in these experiments after etherization of the animal. Consequently, it was necessary to determine what effect ether anesthesia itself might have on the electrolytes of the cerebral cortex. Cortical specimens were obtained from each of three adult rabbits before and during etherization.

In each case a specimen was removed from the right frontal cortex under local (procaine) anesthesia, the animal was etherized and a specimen was removed from the left frontal cortex. The latter specimen was removed from one rabbit after five minutes of etherization and from the other two after twenty minutes.

After micro-incineration, the postetherization specimens did show slight differences of ash pattern from that of their controls, but these differences were, in each instance, exactly opposite to the effects observed to result from convulsions. The effect of ether was a diminution of intraneuronal ash density, especially of non-nuclear ash, and a similar dimming of the ash patterns of neurone processes. However, changes of neuronal potassium and sodium could not be detected on crystallographic analysis.

f. Control of muscular activity. Another factor requiring control is that of muscular activity, the most prominent of the manifestations of convulsions. The four adult rats of this series, therefore, were exercised to exhaustion so that pre-exercise and postexercise specimens from their brains could be compared.

A control specimen was removed from the right frontal cortex of each rat under ether anesthesia. The scalp wounds were sutured, and sealed with collodion. Two hours after recovery from the ether each animal was exercised by

a swim of ten to fifteen minutes in a tub of tepid water. Following this the rats were immediately etherized again, and specimens were obtained from the left frontal cortex.

Examination of the micro-incinerated preparations, and crystallographic analysis, revealed no change of total ash or of neuronal potassium or sodium.

g. Topographic control. Control experiments were also desirable for the determination of difference, if any, of electrolyte changes between various areas of the cerebral cortex. In almost all the foregoing experiments only those areas were studied which are readily accessible through trephines of the calvarium, especially the frontal cortex. Also, it was desirable to know what topographic differences of electrolyte distribution might exist in the cerebral cortex of a normal animal.

Six adult rabbits were used. Each of three of these was given six electroshock convulsions in rapid succession, following which craniotomy and removal of cortical disks from various areas were quickly done. The remaining three rabbits were not given convulsions, but similar specimens were removed from their brains. Each of the animals was killed by injection of 40 cc. of air into an ear vein. The areas from which cortical specimens were removed, in each case, were as follows: frontal, parietal, occipital and hippocampal. From each of these areas a specimen was removed from both the right and left sides.

The micro-incinerated preparations revealed only minor, or questionable, differences of electrolyte pattern of corresponding cortical levels of the various specimens from control animals. These differences were in the nature of small variations of density of neuronal ash, and not of ash distribution. There were, however, distinct differences observed among the postconvulsive specimens from each animal. There was a greatly diminished density of neuronal ash in the hippocampal preparations in relation to the appearance of the preparations from the frontal, parietal and occipital areas. No appreciable variations were found among the latter three areas. The decreased density of the hippocampal ash pattern was not accomplished by apparent intraneuronal or extraneuronal changes of ash distribution. It cannot be said whether the appearance of diminished ash in the hippocampal sections represented an absolute or only a relative decrease, since the comparison was with sections from areas known from the preceding experiments to show increased density of neuronal ash following convulsions.

In any event only a minor topographic variation of ash pattern was found among cortical specimens from normal animals, while there was considerable variation in the case of animals which had had convulsions. Crystallographic analyses for sodium and potassium were not done in this series.

COMMENT. As described previously, the changes of electrolytes in the cerebral cortex resulting from experimental convulsions have been controlled carefully with studies of the factors of anoxia, exercise, anesthesia and cortical topography. None of these factors was found to be partly responsible for the effects observed to be associated with convulsions. It is thought, therefore, that such changes of cortical electrolyte directly reflect neuronal activity. Furthermore, the experi-

ments with anoxia and exercise also indicated that trauma to one side of the cortex, as produced in removing the tissue disks, did not induce the changes in the tissues subsequently removed from the cortex of the opposite side. In addition, all histologic procedures in the preparation of sections for micro-incineration and crystallographic analysis were carried out under uniform conditions, the control and experimental material being handled simultaneously.

Objections to the method of micro-incineration have been advanced and are probably valid to the extent that it is not possible to conclude, from the appearance of a micro-incinerated tissue, exactly what the distribution of the electrolyte was in the living tissue. As Gersh (18) pointed out, fixation of specimens for micro-incineration is not instantaneous, but more than that, the fixation itself must cause some alterations of distribution of tissue salts through its shrinking action on protoplasm, if not in other ways. Indeed, this action was evident in all the preparations of these experiments in the form of ash-free lacunae surrounding the cellular ash patterns.

Despite such objections, however, micro-incineration would seem to be a valuable method in experimental histophysiology. In this study it is not concluded that the particles of ash as seen under the microscope were in the same relation to each other as in the living tissue. But it is felt that these methods indicate the general direction of neuronal changes of total "heat-resistant" electrolyte and of potassium and sodium. Changes of cellular hydration were not responsible in themselves for the effects observed since numerous measurements from both micro-incinerated and conventionally stained preparations indicated no change of cellular volume in the postconvulsive specimens.

The finding that potassium apparently is lost from neurones of the cerebral cortex during induced convulsions would seem to supplement the reports of Cicardo and Torino (8, 9) of increased potassium in the cerebral venous blood of animals after convulsions. However, several control analyses of gross cortical specimens from rabbits showed that no change resulted from electroshock or metrazol convulsions in the total potassium or sodium. The described changes must therefore depend on shifts of electrolytes between intracellular and extracellular tissue phases, and not on net loss or gain of these electrolytes by the tissue. The reciprocal relationship shown between neuronal potassium and sodium correlates with work indicating the mutual replaceability of these cations in axoplasm of the giant squid (19). The fact that with convulsions neuronal total ash changed in amount, as well as in distribution, may mean either that there was not apparent equivalence between the amounts of potassium lost and sodium gained, or that other electrolytes accounted for the visible differences of density of the total ash.

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SUMMARY

Studies utilizing the methods of micro-incineration and crystallographic analysis of micro-incinerated preparations indicate that changes of total electrolyte,

sodium and potassium of cerebral cortical neurones accompany or follow experimental convulsions in rats and rabbits. Typically, these neurone changes involved a diffuse increase of total (heat-resistant) electrolyte, a loss of potassium and a gain of sodium. The described changes were detected quite consistently in rabbits given metrazol or electroshock convulsions, and in 50 per cent of rats subjected to audiogenic convulsions. The factors of asphyxia, exercise, anesthesia and cortical topography were not found to contribute to the observed changes. These shifts of electrolytes were found to have a duration of less than three and a half hours (in the case of rabbits given electroshock convulsions) and it is concluded that they are directly associated with neuronal activity.

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THE ACTION OF ADRENALINE ON SPINAL NEURONES SENSITIZED BY PARTIAL ISOLATION[1]

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No uniformity of opinion has been attained regarding the action of adrenaline on the nervous system; this is true both of the peripheral and the central nervous system. To refer only to a few recent investigations, Schweitzer and Wright (17), though mentioning a transient augmentation, found that adrenaline predominantly inhibits spinal reflex activity; Bonvallet and Minz (2) concluded that adrenaline has a depressing effect on the spinal cord but activates the reflexes in a mesencephalic or a thalamic animal; Marrazzi (16) described a depression of synaptic transmission in sympathetic ganglia and a reduction of the electric potentials recorded from the visual and auditory cortex which occurs under the influence of adrenaline; Gantt and Freile (12) studying the effect of adrenaline on conditioned reflexes saw an impairment by it of higher nervous function, while Ivy and co-workers (14) described an analgesic effect of adrenaline. In contradistinction to these results, Bulbring and Burn (4, 5) found that adrenaline increases the stimulating action of acetylcholine on spinal motor neurones, and augments transmission of impulses in sympathetic ganglia. Bulbring and Whitteridge (6) described also an increase of the height of action potentials produced by submaximal stimuli in the nerve under the influence of adrenaline, and Burn (7) speaks of a synergistic action of acetylcholine and adrenaline in the nervous system.

In the meantime, it was shown by Cannon and Haimovici (8), Stavraky (18), and Fisher and Stavraky (10), that spinal neurones become greatly sensitized to chemical stimulating agents after severance of the cortico-spinal tract or removal of the motor cortex. If sufficient time is allowed to pass after the operation, such partially isolated spinal neurones may be selectively stimulated by acetylcholine, acetyl-beta-methyl-choline, strychnine, and other agents when they are introduced into the general circulation in sacrifice experiments, in chronic animals, and in human beings. The technique of sensitization of the spinal neurones by partial isolation seemed most suitable for a study of the action of adrenaline on the central nervous system, and this was made the subject of the present investigation.

METHODS. Two types of experiments were performed. In one, aseptic semisection of the spinal cord was done between T 13 and L 1 vertebrae in 22 cats. Four days to five weeks later, the brain and medulla were pithed under ether anesthesia, artificial respiration induced, sciatic and obturator nerves cut,

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and drills screwed into the two ends of the femurs which were fixed in rigid clamps so that the bones were held vertically and the legs hung freely. The contractions of both quadriceps muscles were recorded by means of threads which were tied symmetrically to the lower ends of the tibiae and connected over pulleys with two identical crank levers of 2:1 magnification pulling against light springs the tension of which with the recording arm horizontal amounted to 10 grams. In 8 experiments, the innominate artery was cannulated and used for the regulation of the blood pressure by a modification of Bayliss' mercury valve (1); the blood pressure was recorded from the left common carotid artery by a mercury manometer, and adrenaline was injected into the aorta through a cannula placed in the left subclavian artery. In experiments in which the blood pressure was recorded, preceding the administration of adrenaline 300 cc. of warm Ringer-Locke solution containing 3.0 to 5.0 mgm. of heparine per kgm. of body weight of the cat were introduced by means of the mercury valve through the innominate artery into the general circulation. The cat was wrapped in absorbent cotton and placed in a box-stand heated by means of an electric bulb. Care was taken to allow complete recovery of the spinal animal from ether anesthesia before the beginning of the experiment; this took 30 to 45 minutes and was judged by the spread of the knee jerk from the control to the semisected side on tapping the patellar tendon on the control side and by the reaction of the preparation to injections of acetylcholine or strychnine. However, in many experiments no drugs other than adrenaline were injected.

In another set of experiments, frontal lobectomized and semidecerebrated cats were prepared and, after complete recovery of the animals from the operation, intravenous injections of adrenaline were repeated once a week or every ten days in a manner similar to that described in the case of acetylcholine (Stavraky, 18). In 8 cats, adrenaline was injected from the beginning, whereas in 4 additional animals sensitization was first assured by weekly injections of acetylcholine over a period of 4½ months, and the action of adrenaline then tested. Two cats were kept for three years before the adrenaline was tried out.

Crystalline epinepherine (BDH) and Parke-Davis' Adrenalin Chloride Solution were used in a 1:10,000 to 1:20,000 concentration, dissolved in mammalian Ringer-Locke solution.

RESULTS. *Effects of adrenaline in spinal preparations.* Quantities of adrenaline ranging from 0.05 mgm. to 0.3 mgm. when injected intra-aortally caused marked contractions of the quadriceps on the side of the semisection of the spinal cord in 20 out of 22 experiments. When a large quantity of adrenaline (0.2 mgm. to 0.3 mgm.) was injected for the first time in the experiment (fig. 1) it often caused a transient relaxation of the quadriceps which was followed by a slow tonic contraction. When the preparation was at the height of its sensitivity these effects were accompanied by a flicker, or even a real contraction of the opposite quadriceps (fig. 2). The response came on usually 6–22 seconds after the beginning of the injection: the relaxation of the quadriceps when it occurred lasted 7 or 8 seconds and was followed by a contraction of 30 seconds to 2 minutes' duration. Small quantities of adrenaline (0.05 mgm. to 0.1 mgm. per

injection) caused a contraction only on the sensitized side. These contractions were less sustained in character and occasionally resembled a clonic movement.

When repeated approximately within 10 minute intervals, injections of adrenaline were effective twice, sometimes three times, the contractions growing less marked with each injection, while the initial relaxation of the quadriceps was usually absent after the first injection. Further injections failed to produce any contractions of the quadriceps on either side in spite of marked effects of adrenaline on the systemic circulation. The sensitivity of the preparation to other forms of stimulation was reduced by the injections of adrenaline; however, the

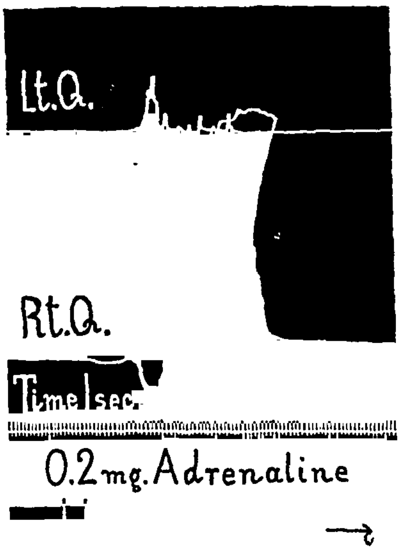

Fig. 1.

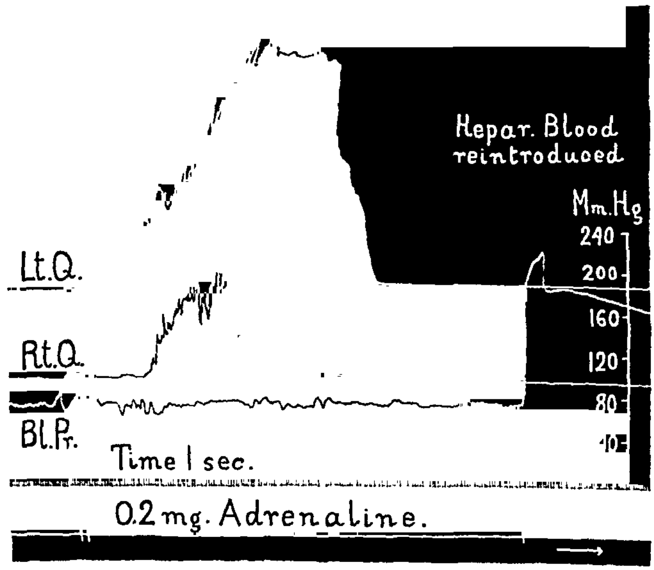

Fig. 2.

Fig. 1. Effect of adrenaline with uncontrolled blood pressure: An intra-aortal injection of adrenaline in a spinal cat 8 days after right semisection of the cord. NOTE initial relaxation of the quadriceps followed by a contraction, both occurring on the semisected side. This is typical of a pronounced effect usually seen on the first injection of adrenaline. (Lt. Q.—Left quadriceps, Rt. Q.—Right quadriceps.)

Fig. 2. Effect of adrenaline with blood pressure stabilized by means of a mercury compensator: An intra-aortal injection of a solution of crystalline adrenaline in a spinal cat 21 days after left semisection of the cord. This is the second injection of adrenaline in the experiment; no other drugs were introduced excepting 300 cc. of heparinized Ringer-Locke solution. Note absence of initial relaxation of the quadriceps.

knee jerks, though reduced, were usually present and the injections of acetylcholine and other chemical stimulating agents evoked a response from both quadriceps muscles. The effectiveness of adrenaline could be greatly augmented, or temporarily restored after its stimulating action wore off by injections of strychnine (0.03 mgm. to 0.15 mgm. per kgm.), or cocaine (5.0 mgm. to 10.0 mgm. per kgm.), and occasionally, camphor (5.0 mgm. to 10.0 mgm. per kgm.). Picrotoxin (0.03 mgm. per kgm.) was also seen to have a beneficial effect on it.

There was no definite relation between the muscular response and the changes in blood pressure caused by adrenaline. In experiments in which the latter was recorded, the contractions of the quadriceps were seen sometimes to precede the

rise of blood pressure evoked by adrenaline, and on other occasions to come on late, i.e., when the blood pressure was declining. As seen in figure 2, in experiments in which the blood pressure was controlled by a mercury compensator, the contractions of the quadriceps came on in the absence of any appreciable change in blood pressure. Furthermore, after the first injection of adrenaline, raising of the blood pressure by rapidly reintroducing the blood from the mercury compensator, or by an infusion of Ringer-Locke solution produced only a slight occasional twitch of the quadriceps or had no effect whatsoever. In spite of this, a second injection of adrenaline caused a marked contraction of the quadriceps (fig. 2). Late in the experiments, when the blood pressure was low, and the condition of the preparation was deteriorating, the contractions of the quadriceps which occurred after injections of adrenaline were seen to be related to the rise of blood pressure, but in view of the failing circulation, this could not be regarded as a normal response.. The possibility of a direct effect of adrenaline on the muscles was excluded by a temporary clamping of the aorta at the level of the bifurcation of the iliac arteries: injections of adrenaline in such experiments were found to produce prompt contractions of the quadriceps. On the other hand, section of the femoral nerve in the presence of an intact blood supply to the muscles not only precluded the response, but when carried out during a contraction of the quadriceps following an injection of a very large quantity of adrenaline (0.3 mgm.) which produced a bilateral contraction of the quadriceps, caused the muscle to relax immediately, while the contraction on the other side went on for the usual length of time. Nembutal (20.0 mgm. to 30.0 mgm. per kgm. of body weight—0.3 cc. to 0.5 cc. of Abbott Solution) administered intra-arterially abolished the response of the quadriceps muscles evoked by adrenaline and even when diluted 100 times Nembutal greatly depressed the response, the depression being more marked on the semisected side. Ether anesthesia had a similar effect.

Effects of adrenaline in chronic animals. Intravenous injections of adrenaline into unanesthetized semidecerebrated or frontal lobectomized cats led to asymmetrical responses which usually became apparent 2 to 4 weeks after the operation and were most prominent toward the 5th month. In two cats, injections of adrenaline yielded characteristic results three years after the operation. Quantities of adrenaline under 0.03 mgm. per kgm. of body weight caused a predominant dilatation of the contralateral pupil which was often followed by a slight but definite extensor rigidity in the contralateral extremities, the latter persisting 2 or 3 minutes after the injection. Injections of 0.03 mgm. of adrenaline per kgm. of body weight had the same effect on the pupils but, as shown in figure 3, caused initial flexion and weakness of the contralateral extremities: this was supervened in 1 or 2 minutes by extensor rigidity which was more pronounced and lasted longer than that observed after smaller quantities of adrenaline. Injections of 0.1 mgm. of adrenaline per kgm. of body weight caused a marked weakness of the contralateral extremities which lasted about 2–4 minutes. If the animal was made to walk during this stage, it often had difficulty in overcoming the weakness in the front paw and started out with a limp which rapidly improved with use of the extremity, but the cat walked raising the contralateral extremities high in the air. Regardless of whether the animal used the extremities or not,

the stage of flaccidity and weakness was followed by a 2 to 4 minute period during which the contralateral limbs became hypertonic. In this stage, the front paw was usually held out rigidly in front of the body and the head turned to the operated side. In the case of large doses of adrenaline, it was occasionally noted that in the early stages of the reaction, particularly on exposure to bright light, the pupil on the side of the operation was larger than the contralateral one; towards the end of the response this reversed itself and the pupil on the opposite side became markedly more dilated than the one on the side of the operation.

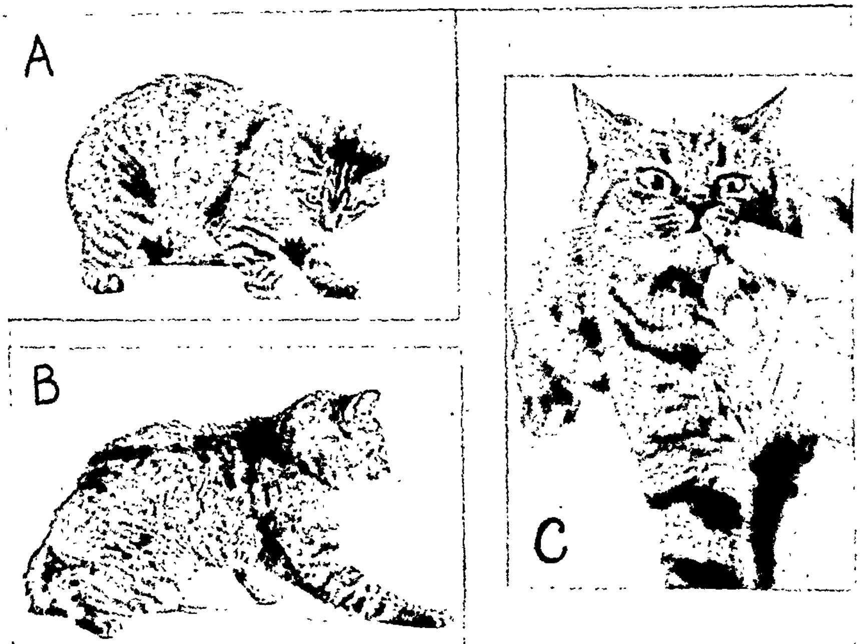

Fig. 3. An intravenous injection of 0.03 mgm. of adrenaline per kgm. of body weight in a cat three years after the removal of the left frontal lobe (including the motor cortex).
A—2 minutes after the injection—showing weakness of the right extremities.
B—5 minutes 30 seconds after the injection—showing rigidity of right extremities.
C—6 minutes after the injection—showing inequality of the pupils: the right being larger that the left. NOTE the forward thrust of the rigidly extended right front paw.

Blanching of the mucous membranes, salivation, occasional vomiting, and some slight hyperpnea accompanied the response which lasted in sensitive animals up to 10 or 12 minutes.

After the visible manifestations of the injection wore off, successive administrations of adrenaline were almost ineffective. This reduction in the sensitivity of the animals to further injections lasted as long as a week or 10 days. The described pattern of the response was observed in most of the injected animals: however, in some, the inhibitory effects of adrenaline predominated, while in others, rigidity came on quickly and was quite marked. Though slight spasticity and even mild convulsions have been described in normal animals on injection of adrenaline (Ivy et al., 14) in the present investigation in the cat, with the quantities of adrenaline employed, even when the motor effects were most

pronounced, they were predominantly unilateral, occurring on the side of the body opposite to the operation and were most marked in the forelimb.

Effects of adrenaline after acetylcholine. As the motor manifestations of the injections of adrenaline were in the second stage of the effect not unlike those seen after injections of acetylcholine (Stavraky, 18), it seemed interesting to see what effect acetylcholine would have on the action of adrenaline. In 4 cats, acetylcholine was injected once a week until the sensitization was fully developed and on the 5th month after the operation adrenaline was tried out. All the excitatory effects of the injections of adrenaline were most pronounced in these animals; quantities of 0.05 mgm. to 0.1 mgm. of adrenaline per kgm. of body weight produced quite asymmetrical dilatation of the pupils, vomiting, extreme blanching of the mucous membranes and a very marked hyperpnea. Then, usually 6 minutes after the injection, a sudden rapid thrust of the contralateral front paw and turning of the head to the operated side, or even a general unilateral contortion similar to the one occurring in the later stages of the acetylcholine convulsion took place. In one cat in which both frontal lobes were removed, 0.07 mgm. of adrenaline per kgm. regularly produced a severe generalized convulsion with typical erection of hair, curving of the back, and throwing of the front paws over the head.

Effects of acetylcholine after adrenaline. In a number of experiments simultaneous injections of acetylcholine and adrenaline were tried out, but more clear-cut effects were obtained when adrenaline preceded the injections of acetylcholine. Injections of adrenaline under 0.03 mgm. to 0.05 mgm. per kgm. of body weight were followed in 30 seconds or so by 0.05 mgm. to 0.1 mgm. per kgm. of acetylcholine. In this combination, adrenaline counteracted, or reduced in severity, the initial generalized convulsion evoked by acetylcholine, but accentuated and markedly prolonged the secondary contortion. As described in a previous article, in frontal lobectomized or semidecerebrated cats, injections of acetylcholine caused unilateral contortions which occurred on the side of the body opposite to the removed part of the brain (Stavraky, 18). In excessively large quantities, adrenaline appeared to have a depressing effect on the action of acetylcholine, particularly if the two agents were injected together.

Discussion. The results presented above show that an injection of adrenaline produces a short initial depression of the partially isolated neurones of the spinal cord followed by a marked excitation and later by a more prolonged residual decrease of their irritability. The sequence of effects brought about by adrenaline in these experiments is not unlike that described by Schweitzer and Wright (17) in their study of the effect of adrenaline on intact spinal neurones; however, in their experiments the excitatory effect of adrenaline was not marked, and could be determined only indirectly by its action on the knee jerk. Sensitization of the spinal neurones by partial isolation or "denervation" increases the excitatory effect of adrenaline upon them to a degree where adrenaline actually produces muscular contractions which originate on the sensitized side, thus bringing out with great clearness the excitatory phase and allowing for its analysis.

As rapid circulatory readjustments, particularly elevations of blood pressure, are known to cause neuronal discharges, the pressor action of adrenaline had to

be excluded as a stimulating factor; this was done by stabilizing the blood pressure with a mercury valve. Furthermore, the stimulating action of adrenaline could not be satisfactorily attributed to its effect on the blood vessels of the spinal cord itself. Forbes, Finley and Nason (11) studying the effects of adrenaline on intracranial blood vessels saw a contraction of the pial arteries on local application of adrenaline, dilatation on intravenous injection, and either effect on intra-arterial injection of adrenaline. They concluded that the rise of blood pressure evoked by adrenaline may counteract the constriction of the intracranial blood vessels, but when it is absent the vessels are capable of constriction. According to these data it is possible to surmise that in the present experiments, particularly in those in which the rise of blood pressure was excluded by means of the mercury compensator, intra-aortic injections of adrenaline may have caused a vaso-constriction of the spinal blood vessels with a resultant asphyxiation of the nerve cells, which could lead to their stimulation. However, in experiments in which the mercury valve was not used, adrenaline caused even more marked contractions of the quadriceps in the presence of rises of blood pressure. Results analogous to those seen on spinal preparations were observed when adrenaline was injected intravenously into frontal lobectomized or semidecerebrated animals. Under these experimental conditions adrenaline should have caused an elevation of the blood pressure and a consequent increase of the circulation in the central nervous system, but in spite of this it produced a marked rigidity in the extremities on the side opposite to the operation.

If the vascular changes do not seem to be able to account for the stimulating action of adrenaline, there is a possibility that other factors of an indirect nature come into play under its influence. It was shown by Hebb and Stavraky (13), and by Langstroth, McRae and Stavraky (15) that adrenaline greatly increases the permeability of glandular cells to various constituents of the blood: a similar sudden increase of permeability of the spinal neurones to constituents of the surrounding medium or to specific components stored at the synaptic junctions of the neurones may be responsible for the stimulating effect of adrenaline. Then Torda and Wolff (19) found that, in vitro, adrenaline accelerates the synthesis of acetylcholine. Isolated neurones are exceptionally sensitive to this agent (Cannon and Haimovici, 8, and Stavraky, 18) and it is conceivable that an acceleration of the synthesis of acetylcholine at synaptic junctions may account for the stimulating action of adrenaline. Possibly both an acceleration of the synthesis of acetylcholine and a more rapid diffusion of it due to an increase in permeability may take place at the same time. However, an altogether different mechanism of the action of adrenaline may be involved.

Cannon and Haimovici (8) saw under the influence of the semisection of the spinal cord, some increase of the sensitivity to acetylcholine of the quadriceps muscle itself; this was not so in the case of adrenaline. Section of the femoral nerve instantaneously abolished the contraction of the quadriceps muscle evoked by adrenaline, while with the nerve intact, and after the abdominal aorta had been clamped, thus preventing adrenaline from reaching the muscle, adrenaline still produced a contraction of the quadriceps.

Of interest is the fact that even traces of nembutal and ether reduced or abol-

ished the excitatory effect of adrenaline. It is postulated by Bremer (3) that nembutal blocks synaptic transmission in the spinal cord. Eccles (9) too, suggests a specific action of nembutal on the synaptic regions of spinal neurones and on the stability of neuronal membranes as possible causes of a nembutal block. This may account for the abolition of the excitatory action of adrenaline by nembutal in the present experiments. However, the action of nembutal similarly to that of other anaesthetics could be due to a depression of the metabolism of the sensitized neurones. In view of the extreme ease with which the excitatory effects of adrenaline on neurones sensitize by partial isolation are abolished by anesthetics it is quite likely that the action of adrenaline on intact neurones would be affected by them in a like manner. This may explain why Marrazzi (16), who carried out his observations on the nerve cells while the latter were under the influence of nembutal and other anesthetics, saw only a depressing effect of adrenaline upon them, whereas Bulbring and Burn (5), working mostly on perfused preparations in which the effect of an initial anesthetic had time to wear off, were able to detect the stimulating effect of adrenaline in experiments otherwise often not unlike those of Marazzi.

The bringing out of the excitatory effect of adrenaline in chronic animals by injections of acetylcholine carried out over a long period of time is quite remarkable. Noteworthy, too, is the increase and prolongation of the action of acetylcholine which takes place when the latter is superimposed on the action of adrenaline. It is believed that these observations fall in line with the facts reviewed by Burn in an article on "The relation of adrenaline to acetylcholine in the nervous system" but as no analysis of the phenomenon was attempted at the present time a discussion of these observations seems unwarranted. Most intriguing is the great reduction and even absence of the excitatory response to adrenaline after the first two or three injections. In chronic animals this was even more marked than in spinal preparations and the depression caused by adrenaline was seen to persist for several days, the time coinciding with that indicated by Gantt and Freile (12) in their investigation of the effect of adrenaline on conditioned reflexes.

While the inhibition produced by adrenaline in this study, though increased by denervation, compares adequately with that seen by other investigators, it is the stimulating action of adrenaline which is predominantly brought to the fore by partial isolation of the spinal neurones. With this in mind, and in spite of the fact that the quantities of adrenaline employed are of pharmacological rather than of physiological significance, it is tempting to suggest that in certain instances of increased irritability adrenaline may play a part in the nervous system which is consistent with the emergency theory of function of the adrenal glands.

SUMMARY AND CONCLUSIONS

1. Sensitization of spinal neurones by partial isolation markedly increases their susceptibility to injections of adrenaline and allows the various stages of the action of adrenaline to be observed both in sacrifice experiments on spinal cats, and in chronic frontal lobectomized or semidecerebrated animals.

2. Injected intra-arterially or intravenously in quantities ranging from 0.05 mgm. to 0.3 mgm. per injection, adrenaline, after an initial transient depression produces marked excitation of the sensitized neurones, this being followed again by a more prolonged decrease of irritability.

3. These effects of adrenaline are not dependent on blood pressure changes and, as far as can be judged, are not related to any action of adrenaline on the blood vessels of the central nervous system.

4. In spinal preparations, the stimulating action of adrenaline is augmented by cocaine, strychnine, and other convulsant agents, but is abolished by nembutal and by ether anesthesia. In chronic animals, the stimulating action of adrenaline can be facilitated also by repeated injections of acetylcholine, while adrenaline in turn can, in appropriate quantities, increase and prolong the action of acetylcholine.

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THE ACTION OF INSULIN AS INDICATED BY DEPANCREATIZED HERBIVORA

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Our knowledge of experimental diabetes has been derived largely from observations on the depancreatized dog and cat. These two species have been popular laboratory test animals and the surgical removal of the pancreas in them offers little difficulty. It is interesting then that our concepts of diabetes and of the action of insulin should be so greatly influenced by the observations on these two carnivorous animals. In the metabolism after pancreatectomy one would be justified in supposing that the dietary habit of the animal might play an important rôle and that one should give as much weight to observations on depancreatized omnivora and herbivora, as to those on carnivora. Collip, Selye, and Neufeld (1), Chapman and Fulton (2), and Mirsky, Nelson, and Elgart (3), have studied monkeys after pancreatectomy and shown that the results are quite different from those on the cat and dog. Lukens (4) has made several observations on the depancreatized goat and found a mild type of diabetes. Recently Mirsky, et al. (5) have reported a marked difference in the diabetes of carnivorous and herbivorous birds after pancreatectomy.

The depancreatized herbivora should be ideal for studying the action of insulin, since they can be maintained for many days in a steady state without the administration of insulin. It is easy to obtain adequate periods with and without insulin in which to determine the changes which result from the addition of this hormone. Such has not been possible with the depancreatized carnivores; without insulin they do not assume a steady state even for as long as one day.

The distinctive metabolic characteristics of carnivores when depancreatized and not given insulin are: 1. The inability to maintain body weight on a carbohydrate diet. 2. During fasting a production of glucose by the liver that is greater than the utilization of the tissues, so that glucose is continually excreted in the urine. 3. Large production of ketone bodies during fasting and usually even when given food.

In the attempt to explain these findings, certain writers (6) in the past had developed the theory that insulin is necessary for the oxidation of glucose. Others (7) explained the results on the basis of the theory that insulin inhibits over-production by the liver of glucose from protein and fat. It is pertinent to consider these theories in connection with the findings in herbivora from whom the pancreas can be removed as completely as is the case with diabetic carnivores.

METHODS. The surgical procedures required for pancreatectomy in the rabbit have already been described (8). Instead of the three stage operation which was used at that time, it has been found possible to remove the entire pancreas

in one stage, providing the rabbit has been fasted about four days. Rabbits weighing about two kilos are best suited for this purpose. The diabetic rabbit which is not receiving any insulin is subject to staphylococcus infections, death usually resulting from extensive collections of pus radiating from the surgical wounds causing peritonitis, subphrenic abscesses, or empyemas. Therefore, it is desirable to start injections of insulin as soon as the animal is eating well. The amount of insulin administered must be carefully controlled to prevent hypoglycemia and death, particularly when using protamine zinc insulin. Plain insulin may be used quite safely, but requires frequent injections. Rabbits about one week after the operation which are eating well, require six to twelve units of protamine zinc insulin daily to keep glycosuria at a low level.

Pancreatectomy in the goat. (The animal should be fasted for 24–36 hours before operation in order to have the stomach as small as possible.) Intravenous nembutal, 0.5–1.0 grain per 5 lbs. body weight was used as a basic anesthetic. During the operation a small amount of ether was given by mask to keep the animal at the desired level of anesthesia. After opening the abdomen through a mid-line incision, the great omental sac was opened, the large stomach retracted, and the pancreas exposed in the region of the portal vein. Usually the removal of the gland was begun at the head end and consisted essentially of careful dissection along the portal vein to prevent sectioning of blood vessels. Smaller veins coursing through the pancreas from abdominal viscera and vessels supplying the pancreas were, of course, ligated as found. Location of the common bile duct was accurately determined to prevent possible ligation. The abdominal incision was closed by several layers of silk sutures. No outside dressing or support was required. Recovery was usually rapid and within a week or two the depancreatized goat was ready for experimental observation. Administration of 10–20 units protamin in insulin daily was used postoperatively to aid recovery processes.

RESULTS. *Maintenance of body weight on carbohydrate diet.* Tables 1 and 2 give the weight changes for the rabbit and goat respectively when on a hay and alfalfa diet. There is a weight loss in all cases when not given insulin and when insufficient insulin is given. The results indicate that these animals are not quite able to maintain a steady state on a carbohydrate diet. The depancreatized rat can do so (9). This latter preparation can maintain a constant weight by virtue of a prodigious consumption of a diet of high sugar content; on a high fat diet it can maintain itself with ease. It is likely that the rabbit and goat could also maintain a steady state if they could consume proportionally large amounts of carbohydrate in such concentrated form as is possible with the rat. It is difficult to carry these animals for a long period without feeding bulky food such as hay or alfalfa. We were unsuccessful in finding any high fat diet which these animals could be induced to take.

We were able, in some animals, to administer a high sugar intake either by feeding a sugar solution by stomach tube or by teaching the animal to drink the solution in large quantities. The following observations are suggestive in this connection. A depancreatized goat was fasted for several days, the blood sugar

fell and glycosuria ceased. The introduction of 100 grams glucose (10 per cent solution in water) into the stomach caused an elevation of the blood sugar to over 200 mgm. per cent and glycosuria. After 48 hours the blood sugar returned to normal, but only 40 per cent of the administered sugar was found in the urine

TABLE 1

Weight changes in diabetic rabbits on hay and alfalfa diet

RABBIT	DURATION OF PERIOD	AVERAGE DAILY INSULIN UNITS	WEIGHT CHANGE FOR PERIOD	
			Loss	Gain
	days		*gm.*	*gm.*
A	9	0	100	
	12	6.3 PZI		395
F	12	0	150	
	6	5 PZI		30
G	9	1.7 plain	85	
	6	4.6 PZI		50
H	30	0	490	
	7	3.5 PZI		170

TABLE 2

Weight changes in diabetic goats on hay and alfalfa diet

GOAT	DURATION OF PERIOD	AVERAGE DAILY INSULIN UNITS	WEIGHT CHANGE FOR PERIOD	
			Loss	Gain
	days		*kgm.*	*kgm.*
1	39	0	0.4	
	7	36 PZI		3.1
2	26	1.3 PZI	0.1	
3	4	37 plain	0.5	
	12	9.2 PZI		0.3
	30	12.0 PZI		3.1
4	39	0	0.7	
5	17	0	0.5	

Similar experiments with sucrose showed a utilization of 60–70 per cent. In another series of experiments in which the goat was fed alfalfa and given sucrose solutions to drink, carbohydrate utilization ran around 75–80 per cent.

Tables 1 and 2 show that these animals will gain in weight if they are given adequate insulin and the activity of it is spread out by protamine. Plain insulin does not effect this since the duration of activity of an ordinary dose is only

3 to 6 hours (10) and although the animal might gain some weight during this period it would probably be lost again during the time the animal would be without insulin activity.

Gluconeogenesis during fasting. To study this factor, urine nitrogen and sugar were determined during fasting in depancreatized and in normal control goats. Tables 3 and 4 give these findings. During the first few days of the fast, the diabetic animals excrete the excess sugar that is present in their bodies at the time food is withdrawn (this includes considerable carbohydrate in the gut that takes some time to be assimilated). Thereafter the tissue utilization keeps pace with the new glucose formation so that the blood sugar level is normal without

TABLE 3

Diabetic goat no. X—fasting

DAY	WEIGHT	URINE VOL. CC. VOIDED	URINE SUGAR	URINE N GM. PER KGM. PER DAY	NOTES
	kgm.		*gm.*		
1	17.7	1050	15.9	0.39	
2	16.7	175	0.0	0.12	
3	15.6	75	0.0	0.07	
4	14.5	130	0.0	0.18	
5–6	13.6	290	0.0	0.25	Died

TABLE 4

Goats—fasting urinary nitrogen

NORMAL			DIABETIC		
Animal	Days of fast	Urinary N gm. per kgm. per day	Animal	Days of fast	Urinary N gm. per kgm. per day
A	3–6	0.07	X	5–9	0.101
B	6–8	0.168	X	4–7	0.09
C	2–10	0.146	C	1–5	0.125
Average		0.128			0.105

any urinary glucose excretion. The urinary nitrogen of the diabetic animals parallels that of the controls, decreasing during the first two or three days, then staying relatively constant for several days and finally rising again as the fat stores of the body become exhausted. The diabetic animals showed this secondary rise sooner than controls because they usually had low fat stores.

Over-production of ketone bodies. Occasional tests for acetone in the urine were carried out. These were usually negative when the animal was receiving insulin and were never strongly positive when the animal was not getting insulin even when fasting. Five diabetic goats were followed in this regard. During the fasting period of one goat, ketone bodies disappeared entirely from the urine.

Discussion. Present day theory of insulin action as presented in modern textbooks is viewed either as concerned with oxidation of glucose or with preventing over-production of this substance. Neither of these receives support from results in the depancreatized herbivora. Indeed, if this type of animal had been that which the first investigators had used instead of the dog, it is unlikely that such actions would ever have been suggested. The amount of carbohydrate which we have found the goat and rabbit able to utilize is sufficient for the metabolic needs of the animal, so that the lack of insulin does not lead to any insufficiency of glucose oxidation. Other means of disposal of glucose than that mediated by insulin must be present in the body. The tissue glucose utilization of the fasted animal supplies only some 20 per cent of the total fuel needs (11). This utilization is increased by feeding (12). Our results indicate that in the depancreatized goat fed carbohydrate this is increased to such a degree that nearly all the energy needs of the body are supplied by carbohydrate. This is accomplished without the aid of insulin and suggests that there are other hormones regulating "immediate" glucose utilization.

On the other hand, there is no evidence of over-production of glucose as is apparent from the fact that the blood sugar of the depancreatized goat becomes normal when the animal fasts. The fact that this animal did not gain weight when not given insulin and since it did so when given insulin, emphasizes the important function of insulin in promoting the storage of carbohydrate. Indeed, in the goat this seems to be practically the only use of insulin: to store food largely as fat at times when the animal has a large intake of carbohydrate. This effect of insulin is its chief action in the depancreatized rat (9). Our results suggest that in the absence of insulin, carbohydrate cannot be utilized by the pathway: first by conversion to fat and then oxidation of this fat. It follows that the other hormones regulating glucose utilization mentioned above are concerned with the direct oxidation of it and not in its conversion to storage substances like fat.

The large difference between herbivora and carnivora after pancreatectomy is of interest. The depancreatized goat and rabbit do not produce an excessive amount of ketone bodies when fasting. In these animals, insulin is not necessary to prevent ketosis. One is therefore not justified in making any general rule for all vertebrates to the effect that insulin is necessary to hold in check an innate tendency to over-produce these substances. Insulin seems to have such a function in carnivora, and hence there must be some special action of this hormone in these animals, probably involving the relationship of insulin to another endocrine. The ketogenic hormone of the anterior pituitary comes to mind in this connection. Could insulin exert its effect in the carnivora by antagonizing the ketone-body producing process under the control of ketogenic hormone? Price, Cori, and Colowick (13) have shown that insulin can reactivate hexokinase previously inhibited by anterior pituitary extract. By analogy we may consider the possibility of a direct action of insulin on the ketone body producing process (rather than an indirect action on the anterior pituitary itself, which would alter the rate of secretion of ketogenic hormone). The relationship is not a simple one,

since increased insulin activity may lead to hypoglycemia which can cause ketosis (14).

In a similar fashion we may say that insulin is not universally necessary to combat a tendency to excessive breakdown of tissue protein, since the fasting depancreatized goat shows a nitrogen catabolism which is normal. In the fasting depancreatized carnivora, insulin would likely have its action in combating the excessive protein catabolism by opposing some hormone such as that found in adrenal cortex extract.

SUMMARY

The depancreatized goat and rabbit are proper preparations for the study of insulin action in the body. This is because they can maintain a healthy steady state when not given insulin, so that with them suitable control periods can be obtained that can be compared with periods during which insulin is administered. Such a contrast cannot be obtained with the depancreatized dog or cat. If insulin is withheld from the depancreatized herbivora, there is no lack of utilization of glucose for current metabolic needs, and during fasting, there is no excessive breakdown of body protein or over-production of ketone bodies.

In depancreatized carnivora, insulin is required to combat these disturbances: this indicates that other hormones are involved in the production of these conditions in these species.

The only apparent function of insulin in herbivora is that of mediating storage of carbohydrate.

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FURTHER STUDIES ON STREAMLINE BLOOD FLOW IN THE ARTERIES OF THE CAT[1]

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In a previous paper (1) two of the present authors described a simple technique for studying the blood flow pattern in the arteries of the dog and cat. Evidence was given for the streamline nature of the flow in the arteries, and the probable streamline nature of the flow at the aortic orifice. The present paper represents a continuation of that study. Opportunity will also be taken to answer certain theoretical criticisms made of the earlier paper.

METHODS. Observation of the blood flow in the lower abdominal aorta of the cat has been improved by the use of a lucite rod, flattened at the end, and bent into the form of a trench, in which rests a glass segment inserted into the blood vessel. Light from a ribbon filament Bausch and Lomb microscope lamp was transmitted down the lucite rod, yielding better illumination of the blood flowing through the glass segment than had previously been achieved.

Measurement of the distribution of injected India ink in the blood of various arteries of the cat was accomplished by the use of a Coleman spectrophotometer. Samples of blood were drawn simultaneously from different arteries while ink was being injected at some point upstream, and after suitable dilution with distilled water, the transmissions of the several blood samples to light of 6000 Angstrom units were compared by reference to a distilled water standard. It was anticipated that the streamlining of the ink flow might be reflected in unequal distribution of the ink to main and tributary arteries, and such proved to be the case.

RESULTS. When ink is caused to flow from a fine needle into the lumen of a glass segment inserted into the lower abdominal aorta, it assumes the form of a filament which sweeps down the blood vessel. This filament is formed whether the tip of the needle is pointed upstream or downstream. The formation of the filament clearly reflects the streamline nature of the blood flow. Individual particles of ink do not exhibit radial motion except under conditions of obvious turbulence, such as when a thrombus forms in the glass segment, resulting in violent eddy formation. It should be emphasized that under the conditions of

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these experiments (deep anesthesia, open chest) the dynamical state of the blood flow in the lower abdominal aorta is very far indeed from being at a critical point as regards transition from laminar to turbulent flow.

It was possible, by this method of direct observation, easily to confirm the finding of Broemser (2) that there is a retrograde movement of the blood in the abdominal aorta during a brief portion of the cardiac cycle. Individual particles of ink show this reversal in direction of movement. No attempt was made, however, to relate closely the period of retrograde movement to a particular part of the cardiac cycle, nor to determine its exact duration. It is remarkable that even during this retrograde phase of movement of the blood, the particles of ink still show no radial motion, i.e., turbulence does not occur to any appreciable degree.

Table 1 shows the distribution of ink in various arteries after injection of the ink at some point upstream. The figures are percentage transmission of the blood samples to light, against a reference standard. A *lower* figure therefore represents a *higher* concentration of ink. The data are based upon observations in nine cats. The control figures refer to blood samples taken from various arteries after ink had been injected, and ample time allowed for re-circulation. The column of differences has been added to facilitate comparison of the several sets of data.

Discussion. A statistical analysis of the data in table 1 is presented below. The test used is that designated number 194 in Neyman and Pearson's *On the problem of the most efficient tests of statistical hypotheses* (3). The data on the coeliac artery and the abdominal aorta above the renals have not been used in the following presentation.

We have two supposedly normal populations with true means α and standard errors σ, say Pop.$_1$ with α_1 and σ_1, and Pop.$_2$ with α_2 and σ_2, and a sample of n_1 from Pop.$_1$ and of n_2 from Pop.$_2$. We may test (the null hypothesis) $\sigma_1 = \sigma_2$, with the common value unspecified. As allowable alternatives α_1 and α_2 can have any values, equal or not, and $\sigma_2 \geq \sigma_1$.

Pop.$_1$ is the control group ($n_1 = 5$).

Pop.$_2$ will be the various experimental groups.

Let

$$\bar{x}_i = \frac{1}{n_i}\sum_{j=1}^{n_i} x_{ij}$$

$$s_i^2 = \frac{1}{n_i}\sum_{j=1}^{n_i} (x_{ij} - \bar{x}_i)^2$$

and

$$u = \frac{n_2 s_2^2}{n_1 s_1^2 + n_2 s_2^2}$$

where $\bar{x}_i$ is the mean, i refers to the particular population, x_{ij} are the j differences in the i population, s_i is the standard deviation of population i, and u is the test statistic.

TABLE 1

Inequalities in the arterial distribution of injected ink

SITE OF INJECTION	PER CENT TRANSMISSION OF BLOOD FROM			DIFFERENCES
	Abdominal aorta below renals	Renal artery	Coeliac artery	
Left ventricle	32.0	29.0		3.0
	58.5	59.0		0.5
	49.5	51.5		2.0
	62.5	63.5		1.0
	27.5	28.5		1.0
Root of aorta approximately 1 cm. beyond aortic valve	45.0	41.0		4.0
	30.0	32.0		2.0
	23.5	19.0		4.5
	31.5	29.5		2.0
	9.0	11.0		2.0
	5.0	6.0		1.0
	39.0	36.0		3.0
	20.0	19.0		1.0
	8.5	5.5		3.0
	47.0	20.5		26.5
	30.0		28.5	1.5
Descending thoracic aorta	33.5	39.0		5.5
	56.0	56.0		0.0
	52.5	40.0		12.5
	47.0	27.5		19.5
	35.0		34.0	1.0
	23.0	37.0		14.0
Abdominal aorta above renals	51.0	60.5		9.5
Controls	48.5	48.5		0.0
	49.5		50.0	0.5
	53.0	53.0		0.0
	61.5	60.0		1.5
	65.0	64.0		1.0
	71.0	71.5		0.5

Then we reject the hypothesis $\sigma_1 = \sigma_2$ if $u \geq u_\epsilon$ where u_ϵ is determined from the incomplete Beta function:

$$\epsilon = \frac{\int_{u_\epsilon}^{1} u^{\frac{n_2-3}{2}} (1 - u)^{\frac{n_1-3}{2}} \, du}{\int_{0}^{1} u^{\frac{n_2-3}{2}} (1 - u)^{\frac{n_1-3}{2}} \, du}$$

with ϵ the level of significance, which is taken as 0.05, i.e. the probability of rejecting the hypothesis $\sigma_1 = \sigma_2$ *if it is true* is 0.05.

Case 1. Injection into left ventricle.

$\bar{x}_1 = 0.4$	$n_2 = 5$
$n_1 s_1^2 = 2.7$	$u = 0.846$
$n_1 = 5$	$u_{0.05} = 0.865$
$\bar{x}_2 = -0.3$	$pu = 0.064$
$n_2 s_2^2 = 14.8$	

where p_u is the probability of getting a difference in variability as great as that observed if there is no actual difference, no assumption being made about the values of means. $u_{0.05}$ is greater than u, so this is not a significant difference at the 0.05 level, but would be at the 0.10 level.

Case 2. Injection into root of aorta.

$n_2 = 10$	$u = 0.9957$
$\bar{x}_2 = 3.9$	$u_{0.05} = 0.931$
$n_2 s_2^2 = 618.4$	$p_u = 0.0005$

so this is a significant difference at any level.

Case 3. Injection into the descending thoracic aorta.

$n_2 = 5$	$u = 0.9963$
$\bar{x}_2 = 2.5$	$u_{0.05} = 0.865$
$n_2 s_2^2 = 732.5$	$p_u < 0.0002$

so this is a significant difference at any level.

The results of the foregoing analysis may be summarized as follows:

1. When the injection is made just beyond the aortic valve, the differences in distribution of the ink to the lower abdominal aorta and renal artery are statistically highly significant.

2. When the injection is made into the descending thoracic aorta, the differences in distribution of the ink to the lower abdominal aorta and renal artery are statistically highly significant.

3. When the injection is made into the left ventricle, the results are of borderline significance. That is to say, the differences are not significant if a level of significance of 0.05 is used, but are if 0.10 is used.

The data are consistent with the conclusion that the blood flow close to the aortic valve, and throughout the aorta, is laminar, but as we emphasized in our original paper (1), nothing short of direct visualization of the blood flow at every point can decisively establish this point. The borderline results of the ventricular injections perhaps may suggest incomplete mixing in the left ventricle.

Perusal of table 1 will show that it is impossible to predict the exact manner in which injected ink will be distributed to different arteries. This fact may be due to a variety of circumstances, chief of which, probably, are the position of the needle during injection, and the variability in size and angle of branching of the tributary arteries. Thus the renal artery is sometimes large and sometimes small, and may be double. The angle which it makes with the aorta is subject to considerable variation.

Visscher and Hemingway (4) have criticized certain statements made by two of the present authors in an earlier paper (1). These criticisms will be presented in full:

1. "However the above authors fail to state that their calculations are valid only for instantaneous velocities. Actually during the course of a single ejection phase a time-velocity function must be considered, as was pointed out by Katz."

2. "Unfortunately, Ralston and Taylor stated that in calculating cardiac work 'the classical (frictionless flow) formula should be used when the flow is known to be turbulent.' This assertion ignores the fact that when flow is turbulent the energy cost of giving forward velocity is greater than the kinetic energy of forward movement, because in turbulence a large share of the energy expenditure is dissipated in rotational movement. It is precisely because extra energy must be supplied to overcome the increased resistance due to turbulence that conditions that cause turbulence raise the work of the heart. Thus, although it is roughly true that the kinetic energy of forward motion in turbulent flow is approximately $\frac{1}{2}$ mv^2, it is an error to suggest that the work of the heart may be measured by that expression when flow is turbulent."

The first criticism may be answered by stating the fact that in the earlier paper (1) particular reference was made to the more refined integral formula of Katz, it being merely pointed out that since the kinetic energy term in laminar flow is approximately twice that in turbulent flow, the summation implied by the integral must omit the factor $\frac{1}{2}$, since each term of the sum will differ by that same factor.

In the second criticism, certain principles of fluid mechanics have been overlooked. Many years ago the British engineer Reynolds, cited by Bingham (5), showed that the pressure gradient in fluid flow is proportional to the mean velocity raised to some power, this power being 1 for laminar flow and approximately 1.7 for turbulent flow. The pressure-velocity relationships are therefore quite different in laminar compared with turbulent flow. The usual approximate formula for the work of the heart includes both a pressure term and a velocity term, and it is the sum of these which measures the work of the heart. The fact that the kinetic energy term in laminar flow is approximately twice that in turbulent flow therefore should cause no anxiety.

It must be freely admitted that the kinetic energy term in laminar flow is always so small compared with the pressure energy term that it may be neglected. For this reason we do not wish unduly to insist upon the use of the revised formula when the flow is known to be laminar. It is of great importance, however, that the dynamical pattern of the blood flow be kept clearly in mind in studies on the circulation. When Starr et al. (6) formulated the theory of the ballistocardiograph, they used the data of Machella (7) on the blood flow velocities in the aorta of the dog. Machella calibrated his measuring device by determining the rate of volume flow of blood from the vessel and dividing this by the cross-sectional area of the lumen, thus obtaining the mean linear velocity. Under the conditions of the calibration, however, the velocity pattern must have

been very different from that obtaining within the intact vessel. With such a mode of calibration, one could only be justified in saying that the blood flow in the intact vessel behaved *as though* it had a certain mean velocity. The situation is exactly analogous to that occurring in radiation studies, where a radiation known to be heterochromatic is assigned a single "effective" wave-length, on the basis of its having absorption characteristics like those of a monochromatic radiation of that particular wave-length. It is extremely suggestive that Machella noted in his paper that, for a given mean velocity, the cooling powers of blood and water were not at all what one would expect from their respective specific heats, and suggested that viscosity might be an important factor in producing these differences.

SUMMARY

1. Observation of the movement of particles of ink in a glass tube inserted into the lower abdominal aorta of the cat shows that the flow is streamlined under the conditions of the experiments. Broemser's finding of a retrograde movement of the blood during a brief portion of the cardiac cycle is confirmed.

2. Evidence is given that ink injected into the root of the aorta near the aortic orifice, or into the descending thoracic aorta, may be unequally distributed to tributary arteries. These findings are consistent with earlier evidence for laminar flow in the aorta.

3. Certain criticisms of an earlier paper, pertaining to the revised formula for the work of the heart, have been discussed.

4. The importance of recognizing the dynamical pattern of the blood flow in studies on the circulation has been emphasized.

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STABILITY OF PROTHROMBIN[1]

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Clarification of the problem of plasma prothrombin stability was the primary purpose of this study. Evidence is presented indicating that oxalated bovine plasma stored at 5°C. retains most of its prothrombin activity for several weeks. The evidence is based upon prothrombin analyses made by the quantitative 2-stage method of Warner, Brinkhous and Smith (11, 12). It is supported by prothrombin isolation experiments. In addition, stability studies made with purified prothrombin preparations are included which provide substantial evidence that oxalated bovine plasma contains prothrombin stabilizing factors.

A number of investigators (6, 8, 9, 14, 16), all of whom used the 1-stage method of Quick (7) for measuring prothrombin time, have concluded that prothrombin disappears rapidly from blood or plasma which is stored at low temperatures. Page and de Beer (6) found an increased prothrombin time in human plasma stored for 1 hour at 5°C. Quick (8) reports that he has repeatedly found a reduction of prothrombin in oxalated blood when stored for 24 hours in an ordinary refrigerator. The losses occasionally amounted to as much as 50 per cent. Rhoads and Panzer (9) concluded that "bank blood" was no longer a fit source of prothrombin after 3 days at 4°C. Other workers (1, 3), using the 1-stage method, found good prothrombin stability for several days in blood stored at low temperatures.

Reports of prothrombin stability in preserved blood have been made by 3 groups of workers where both the 1-stage and the 2-stage methods of analysis were used. Each group found good prothrombin stability using the 2-stage method. Lord and Pastore (4), using the 2-stage method, found 80 per cent of the prothrombin remaining in bank blood after 9 days at 2 to 4°C. Sixty-one per cent of the activity could still be accounted for at the end of 3 weeks. The 1-stage method showed a 77 per cent loss within 6 to 8 days and 86 per cent at the end of 3 weeks. Ziegler, Osterberg and Hovig (15) reported a decrease with the 2-stage method in the prothrombin titer of banked blood to about 70 per cent of the initial value in 14 days and to 42 per cent in 37 days. In this study they found an increase in prothrombin time with the 1-stage method from 18 to 29 seconds after 37 days' storage. In citrated human blood stored at 3 to 5°C. Warner, DeGowin and Seegers (13) found 70 per cent activity at the end of 3 weeks (2-stage). The other method showed a greater loss but no instances were found in which the prothrombin value fell to less than 40 per cent of the control level during 3 weeks of storage.

It is not logical that the 2-stage method would reveal greater amounts of prothrombin than actually exist. Since the 1-stage method usually indicates

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the lower values after storage it may be presumed that this method is in error because it is dependent upon a number of variables which themselves are altered by storage. Based on this reasoning it may be concluded that prothrombin is relatively stable in blood or plasma under normal storage conditions. It has been possible to support this conclusion with other evidence which is included in this report.

METHODS. *Bovine plasma.* Bovine blood was obtained at the slaughter house where one volume of anticoagulant (1.85 per cent $K_2C_2O_4 \cdot H_2O$ + 0.5 per cent $H_2C_2O_4 \cdot 2H_2O$) was thoroughly mixed with 16 volumes of blood. As soon as possible the plasma was separated by centrifugation and either used immediately or stored at $-30°C$.

Purified prothrombin. The purified prothrombin products used in these experiments were prepared according to the methods described by Seegers, Loomis and Vandenbelt (10).

Imidazole buffer. One and seventy-five hundredths grams of imidazole (Eastman Kodak) were dissolved in 90 cc. of 0.1 N. HCl. The pH was adjusted to 7.25 with concentrated HCl or NaOH and the mixture was then diluted to 100 cc. volume with water.

Prothrombin analysis. The method of Warner, Brinkhous and Smith (11, 12) was used both for plasma prothrombin assay and for the prothrombin products.

EXPERIMENTAL. *Stability of plasma prothrombin.* Four samples of fresh oxalated plasma, each from a different cow, were placed at 5°C. Prothrombin analyses were made at intervals for 80 days. The results are presented in figure 1. Approximately 90 per cent of the prothrombin activity remained after 2 weeks' storage. The prothrombin titer continued to decrease slowly for the remainder of the study. In 2 of the plasma samples 55 and 40 per cent of the activity was present after 80 days of storage, in spite of the fact that some bacterial decomposition had taken place as indicated by strong odors.

Stability of prothrombin in reconstituted dried plasma. Kazal and Arnow (2), using the 1-stage method, reported that drying of plasma from the frozen state does not destroy prothrombin but that it is necessary to reconstitute with distilled water and CO_2 to pH 7.2 in order to obtain a solution in which prothrombin is stable at room temperature for 24 hours. In view of their results and the fact that we have often had difficulty drying purified prothrombin, we conducted the following experiment.

A 25 cc. portion of pooled oxalated bovine plasma was dried from the frozen state and reconstituted the following day with distilled water plus 1 cc. of imidazole buffer. One cubic centimeter of buffer was added to a 25 cc. control plasma sample which had not been dried. The pH of the dried reconstituted plasma plus imidazole was 7.9; that of the unaltered plasma plus imidazole was 7.3. The results (fig. 2) show that there was a loss of approximately 10 per cent of the prothrombin activity in both the unaltered and in the dried reconstituted plasmas after 26 days of storage at 5°C.

Stability of purified prothrombin. Prothrombin product number 8 described by Seegers, Loomis and Vandenbelt (10) is prepared by acetic acid precipitation

from diluted plasma, adsorption on magnesium hydroxide and elution with CO_2. These products are not as stable as native plasma prothrombin although they rarely show much loss in activity at room temperature within 24 hours. However while standing for several days at room temperature small quantities of thrombin appear and then the prothrombin disappears rather rapidly. It has been shown by Mertz, Seegers and Smith (5) that potent thrombin preparations inactivate prothrombin.

Stored plasma as a source of prothrombin. If stored plasma contains as much prothrombin as indicated by the 2-stage analytical procedure it should be possible to separate this prothrombin by suitable isolation techniques. Prothrombin product number 8 has been prepared exactly as described elsewhere (10) from

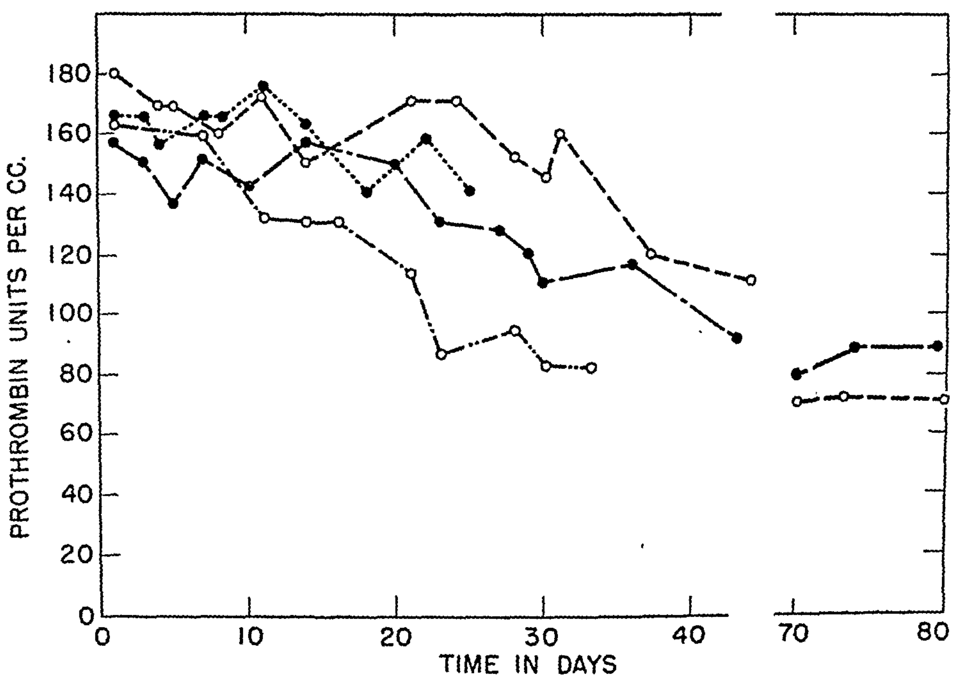


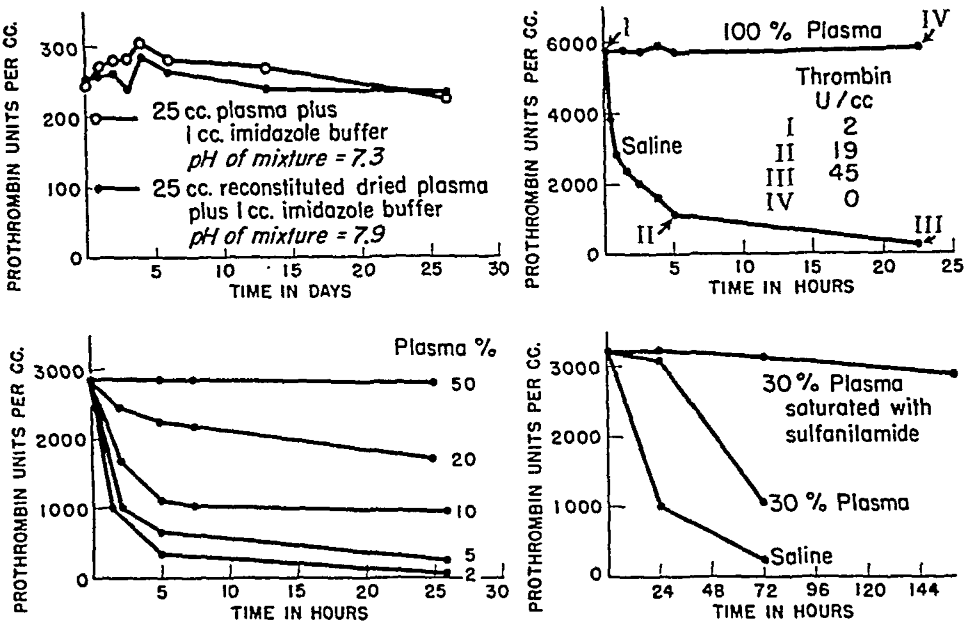
Fig. 1. Stability of prothrombin in 4 samples of oxalated bovine plasma stored at 5° C.

identical quantities of pooled plasma, part of which was frozen immediately after collecting, and the remainder stored at 8°C. for 18 days. The frozen plasma, stored at −30°C., showed no loss of prothrombin activity after thawing, but in the plasma stored at 8°C. the prothrombin concentration dropped to 71 per cent of the original during the 18 day period. As shown in table 1, the two prothrombin products were obtained in almost identical *percentage* yields. This experiment dispels any doubt, not only as to the actual presence of prothrombin in the storage plasma but also shows that the 2-stage method of analysis gave a true indication of the actual quantity present. The stability of both these prothrombin products was the same.

Stability of prothrombin products with high specific activity. When prothrombin product number 8 is further purified by means of $(NH_4)_2SO_4$ fractionation and

subsequent dialysis, as described by Seegers, Loomis and Vandenbelt (10), the resulting product possesses very poor stability characteristics in physiological saline solutions. At room temperature there is usually a rapid loss of activity during the first few hours. The rate of destruction decreases with time so that after several days some activity still remains.

When these purified products are dried from the frozen state and dissolved in oxalated plasma our data indicate that they are as stable as native plasma prothrombin. Therefore, oxalated plasma contains factors which contribute



Figs. 2–5.

Fig. 2. (Top, left) Stability of prothrombin in dried oxalated bovine plasma restored and kept at 5° C.

Fig. 3. (Top, right) Stability, at room temperature, of purified prothrombin with high specific activity.

Fig. 4. (Lower, left) Stability, at room temperature, of purified prothrombin of high specific activity when added to oxalated bovine plasma.

Fig. 5. (Lower, right) Stability, at room temperature, of purified prothrombin of high specific activity when mixed with 30 per cent plasma, and when mixed with 30 per cent plasma plus sulfanilamide.

to the stability of prothrombin. Figure 3 illustrates the stability of such a purified prothrombin product, at room temperature, in 100 per cent plasma and in physiological saline solution. The oxalated plasma solution retained its full activity for 22 hours in spite of the fact that this prothrombin product was exceptionally unstable in physiological saline. The experiment was arranged so that the plasma itself would contribute only about 5 per cent of the total prothrombin activity. It is of interest that small amounts of thrombin appear in the saline solution on standing. Originally the purified product contained 2 units of thrombin per cubic centimeter or slightly less than 0.04 per cent of the

total prothrombin units. This quantity of thrombin increased 8-fold after 5 hours and 22-fold at the end of 22 hours. If thrombin was formed in the presence of the plasma it was inactivated by antithrombin since a detectable amount was not present at the end of the period.

It is known that certain thrombin preparations inactivate purified prothrombin (5). This proposes the question as to whether prothrombin stability in plasma is due only to the action of antithrombin which rapidly removes traces of thrombin impurities. That antithrombin is not the principal stabilizer is indicated by the data summarized in figure 4. The same prothrombin product used in the experiments illustrated in figure 3 was dissolved in saline solutions containing varying amounts of oxalated plasma. There was never any detectable thrombin in the samples containing 5 per cent plasma or more, but, nevertheless, with dilution the plasma became progressively less effective as a stabilizing agent. In the sample containing 2 per cent plasma there were 7 units of thrombin after the 30 hour period at room temperature.

In figure 5 additional stability data are presented. The 30 per cent plasma showed good stabilizing properties for 24 hours at room temperature. At 72 hours the prothrombin activity had decreased to about $\frac{1}{3}$ of the original. After this interval of time there was bacterial decomposition in both the saline and in the 30 per cent plasma solutions. We anticipitated this and added sulfanilamide to one sample. This 30 per cent plasma solution of prothrombin saturated with sulfanilamide was stable for more than 6 days. The additional stabilizing effect probably resulted mainly from suppression of bacterial action. Sulfanilamide alone repeatedly shows a stabilizing effect on saline solutions of purified prothrombin, but its quantitative effectiveness is variable. The results shown on figure 5 are very likely a combined effect of plasma, specific action of sulfanilamide, and sulfanilamide inhibition of bacterial action.

In an attempt to find the prothrombin stabilizing factor in plasma we tested the effect of crystalline bovine albumin on purified prothrombin solutions. It was found that albumin solutions of 0.5 and 5.0 per cent, at pH 7.0, had no stabilizing effects whatsoever.

It is of further interest that $(NH_4)_2SO_4$ appears to act as a stabilizer of purified prothrombin. Table 2 illustrates the effect on 8 different purified products and should be compared with figures 3, 4 and 5. Four per cent $(NH_4)_2SO_4$ solutions of prothrombin (about 10,000 units per cc.) usually show fairly good stability at room temperature.

Prothrombin concentration in bovine plasma. Prothrombin concentrations of 160 to 250 units per cc. were found initially in the different bovine plasmas used in experiments summarized in table 1, figures 1 and 2. These studies were made at different periods of the year and we suspect that there is a seasonal change in bovine plasma prothrombin concentration.

Discussion. It is evident that oxalated beef plasma contains a stabilizing factor or factors for prothrombin. The various reports of poor prothrombin stability in stored blood and plasma as measured by the 1-stage method make it apparent that this method of prothrombin analysis is affected by variables

other than changes in concentration of prothrombin. The 2-stage method provides for complete conversion of prothrombin to thrombin thus eliminating as variables those factors which alter the speed of the reaction. The newly formed thrombin is allowed to clot a standard fibrinogen solution in a constant medium so that the only significant variable in the entire procedure is the prothrombin concentration. Its dependability as a quantitative method for

TABLE 1

Isolation of prothrombin from oxalated bovine plasma

	PLASMA STORED AT 8°C.	PLASMA STORED AT −30°C.
Units per cc. at 0 days	175	175
Units per cc. at 18 days	125	175
Units isolated	245,000	370,000
Per cent yield	65	70

TABLE 2

Stability of prothrombin in ammonium sulfate solutions at 25°C.

PROTHROMBIN PRODUCT	PURITY U/MG TYROSINE	PER CENT $(NH_4)_2SO_4$	pH OF SOLUTION	STABILITY AT ROOM TEMPERATURE
60520	10,900	4	7.0	No loss in 20 hours. 50% loss in 48 hours
60523	15,600	4	7.0	20% loss in 24 hours
61204	13,100	4	6.0	12% loss in 3 hours. 18% loss in 4 hours. 51% loss in 24 hours
70113	13,000	4	6.0	No loss in 3 hours
70114	11,900	4	6.0	35% loss in 3 hours
70115	11,300	4	5.5	17% loss in 4 hours
70120	8,400	4	6.0	22% loss in 3 hours
70203	10,900	4	6.0	27% loss in 2½ hours

prothrombin is demonstrated by the isolation work described above where prothrombin was obtained from storage plasma in a yield predicted possible from the 2-stage analysis of the plasma.

SUMMARY

1. Prothrombin in oxalated bovine plasma is relatively stable at 5°C. In 4 samples of plasma an average of 90 per cent of the prothrombin remained after 2 weeks. In 2 samples approximately 50 per cent was accounted for after 80 days' storage.

2. Oxalated bovine plasma which was dried from the frozen state and reconstituted with buffered distilled water lost only 10 per cent of its prothrombin in 26 days at 5°C.

3. Prothrombin has been isolated in a high yield from bovine plasma stored at 8°C. for 18 days.

4. Purified prothrombin products which are unstable at room temperature are stabilized when dissolved in oxalated bovine plasma.

5. Crystalline bovine albumin is not a prothrombin stabilizing agent.

6. Ammonium sulfate solutions tend to stabilize purified prothrombin.

We wish to thank Parke, Davis and Company for supplying quantities of plasma and for funds for a graduate research fellowship.

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THE EFFECT OF DISCONTINUOUS CHRONIC ANOXIA ON LIVER GLYCOGEN STORES

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Studies of the carbohydrate metabolism of animals subjected to anoxia are of interest because of the changes taking place in the endocrine system under these conditions, and because of the obvious advantages of utilization of an oxygen-rich metabolite during anoxia. One phase of this study is associated with the changes in amounts of liver glycogen before and after exposure to low barometric pressure.

METHODS. The liver samples used for the determinations presented in this report were taken from albino rats (Sprague-Dawley) exposed to 379 mm. Hg (18,000 ft. equivalent) in a large steel decompression chamber[1] one hour per day for nine weeks. During the experimental period they were given dog chow *ad libidum* both at sea level and during exposure to "altitude". At the end of nine weeks the animals were fasted for eight hours and sacrificed under nembutal anesthesia. Liver samples were taken, weighed, and macerated in 95 per cent alcohol to prevent glycolysis. This allowed storage of the samples until it became convenient to perform the glycogen analysis. Glycogen analysis was performed by Sahyun's method (1), the alcohol added earlier being driven off by the initial heating of the latter procedure.

TABLE 1

ANIMAL NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	Avg.
Control % Glycogen...	3.40	2.13	2.65	0.88	2.23	0.69	1.72	0.72	2.31	0.52	2.57	1.92	1.66	1.80
Exposed % Glycogen...	0.35	0.27	1.11	0.33	0.21	0.12	0.22	0.19	0.11	0.09	0.85	0.34	0.24	0.26

RESULTS. The amounts of glycogen present in liver samples of 13 animals previously exposed to discontinuous chronic anoxia and of 13 carefully paired control animals are given in table 1.

DISCUSSION. Evans (2), Lewis, Thorn, Koepf and Dorrance (3) and Nims, Langly and Clarke (4) reported very high liver glycogen contents in rats after exposure to 18,000 and 25,000 feet simulated altitude for single 24 hour periods. This was correlated in their studies with hypertrophy of the adrenal cortex and a corresponding increase in the production of adrenal cortical hormone. It was also shown by Lewis et al. that this phase of high liver glycogen was pre-

[1] Appreciation is expressed for the use, in this study, of a decompression chamber belonging to, and on the grounds of, the National Institute of Health, U. S. Public Health Service, Bethesda, Maryland.

ceded by a period during which there was marked depletion of the carbohydrate reserves. Thorn, Jones, Lewis, Mitchell and Koepf (5) showed, positively for rabbits and also indicated in the case of rats, that repeated daily exposures to low pressure produced an appreciable reduction in liver carbohydrate stores within a 36 day period.

The present paper confirms the observation of Thorn et al. that repeated daily exposure to anoxia brings about the depletion of liver glycogen.

In addition, since the experimental period in the present experiment was long enough (9 weeks) that regression of endocrine changes associated with "acclimatization" had occurred[2], the depletion observed may be interpreted as further evidence of exhaustion of the mechanism imparting hepatic glycogenic function.

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[2] To be reported in a future paper, now in manuscript, of the present author.

30305.

RELATIONSHIP BETWEEN BODY TEMPERATURE AND BLOOD SUGAR IN THE CHICKEN

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The finding that a definite relationship could be established between the body temperature and the arterial pressure in warm-blooded animals (1) as well as in cold blooded animals (2) suggested that other adjustments to body temperature changes might be elicited. To test this possibility the blood sugar of chickens (*Gallus domesticus*) was determined at various body temperatures.

Methods. Twelve white leghorn chickens, 10 to 16 weeks old, were used. The animals were cooled by means of ice packs applied to the body, and warmed by radiant heat from two 150 watt Spot Reflector light bulbs placed about 30 cm. from the body. The rate of body temperature change was about 1°C. per 10 minutes during cooling, and about 1°C. per 8 minutes during warming. Blood samples (0.2 cc) were drawn immediately after each 5°C. body temperature change. Blood glucose was determined using the micromethod of Somogyi (3).

Results. The control blood sugar in our series of chickens averaged 170 mgm. per cent, ranging from 142 to 199. The animals were divided into two groups. Group 1 was cooled first, and then warmed (table 1). Group two was warmed first and then cooled (table 2).

Since 45° and 25°C. were found to be approximately the critical temperatures for survival for the chicken, we did not maintain the animals at these temperatures for more than a few minutes.

Blood sugar levels showed irregular variations in 6 chickens when they were maintained at normal body temperature for periods of two to four hours. The hourly averages for the group in the control period ranged from 177 to 182 mgm. per cent.

It can be seen from table 1 that cooling of the chicken from the normal body temperature of 41.5°C. resulted in a fall in blood sugar which was progressive as cooling of the animal continued, reaching values at 25°C. which were 65 per cent of the control levels. Rewarming of the animals at this time was followed by a return of the blood sugar to almost the control values at 40°C. Continued warming above the normal body temperature levels led to a more rapid rise in blood sugar. At 44°C. the average blood sugar was 26 per cent above control levels. Inspection of the data suggests the presence of a lag in the adjustment of the blood sugar to the changes in body temperature.

In table 2 the lag in adjustment to body temperature can be seen more clearly. Thus, the average blood sugar remained unchanged at the time of sampling

when the animal had been warmed to 44°C. The animal was then permitted to cool spontaneously to its control body temperature. Samples taken at this time showed an increase of 22 per cent. Further cooling by means of application of ice packs to the body resulted in a progressive reduction of the blood sugar until death occurred at about 22 to 23°C. It was noted that these animals were somewhat more resistant to the lethal effects of cooling than those which were cooled without a preliminary warming. Although the blood sugar continued downward with cooling, it can be noted that the average blood sugar

TABLE 1

TEMP.	CHICKEN NO.							AVERAGE
	1	2	3	4	5	6	7	
°C.								
41.5	142	191	199	155	160	152	161	166
35	116	164	135	151		119	146	139
30	76	124	85	135	162	51	151	115
25	72	110	124	107	116	123	96	107
30		142	128	91	143	110	92	118
35		147	142	120	169	129	96	133
40		165	169	136	120	147	114	141
44		295	207			155	179	209

TABLE 2

TEMP.	CHICKEN NO.					AVERAGE
	1	2	3	4	5	
°C.						
41.5	168	179	183	184	170	177
44	219	187	158	139	224	185
41.5	191	188	254	217	228	216
35	181	138	232	237	219	201
30	176	122	180	194	232	181
25	121	120	178	168	227	163
22		134	145			140

values were consistently higher than those obtained in the animals which were cooled without the preliminary warming.

DISCUSSION. These results on the relationship between the body temperature and the blood sugar are in line with our concept that there is an active integration of various aspects of bodily function to changes in body temperature. That at least some of these correlations are a function of the central nervous system is indicated by our finding that the body temperature-arterial pressure relationship in the turtle is destroyed by section of the cervical spinal cord or by destruction of the brain (4).

The blood sugar response to changes in body temperature occurs more slowly than does the blood pressure response. The blood pressure changes occur within

a few seconds after the temperature stimulus is applied, while the blood sugar lags behind the temperature change by about 30 minutes. This would indicate that the blood pressure response is mediated directly by neural mechanisms, while the blood sugar response is dependent upon a more complex, slowly mobilized mechanism. These correlations of blood pressure and blood sugar with body temperature changes merit further work to determine the integrating rôle of the central nervous system.

SUMMARY

1. A direct relationship has been demonstrated in the chicken between the body temperature and the blood sugar, the latter being the dependent variable. The implications of these findings are discussed.

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MEASUREMENT OF THE RESPIRATORY VOLUMES OF LABORATORY ANIMALS[1]

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In the last few years a tremendous amount of study has been directed toward the etiology and pathogenesis of respiratory disease. Quantitative studies of respiratory infection by the cloud chamber method require that the respiratory volume of test animals be known in order to calculate the dose of agent used. Because data in the literature on respiratory volume of laboratory animals are very scanty, a comprehensive study of this subject was undertaken.

METHODS. The various methods used for the determination of respiratory volume may be classed under five different headings: 1, respirograph method (5, 7); 2, valve method (2); 3, air flow methods (1, 8); 4, indirect calculation from O_2 consumption (4, 9); and 5, an acoustic respirograph method (6). Two valve methods and one modified respirograph method have been used in the investigations described in this paper.

A. *Valve methods.* The valves used were of delicate construction utilizing very minute thin rubber discs, which were hinged loosely over the tips of polished glass inlet and outlet tubes.

These valves were then connected directly by means of a glass seal either to a tight-fitting headpiece or to a tracheal cannula.

Collection of air from the outlet valve was accomplished by the two methods shown in figures 1 and 2. When the collection apparatus shown in figure 1 is employed, the expired air enters via the rubber tube attached to the top of the mercury-filled collecting chamber, which may be connected to the water or mercury manometer on either side of the collecting column. By manipulating the stopcock at the bottom of the collecting column, mercury is allowed to fall at a rate which will exactly equalize that of the expired air and maintain pressure within the chamber at exactly zero. By measuring the fall of the mercury column for one minute while the pressure within the chamber is maintained at atmospheric pressure, the respiratory volume per minute may be measured directly. In using this method, the only obstruction to the flow of air is a very slight back pressure from the valves.

The second apparatus for collecting the expired air is shown diagrammatically in figure 2. It is so constructed that no water can flow from the upper siphon jar into the lower jar until an equal volume of air is introduced into the air space above the water in the upper jar. Special precautions have been taken in the design of this apparatus so that the pressure against which the animal must

[1] Data presented on work carried out at Camp Detrick, Maryland, between 1 March 1945 and 1 October, 1945.

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breathe is always constant and very small. By raising and lowering the inlet tube to the upper jar, the pressure for flow of water from the upper bottle to the lower bottle may be adjusted from less than zero to considerably greater than zero. If the critical pressure is less than zero, water will flow of its own accord without an expiratory effort on the part of the animal. If, however, the glass tube is moved downward, the critical pressure becomes positive and water flows from the upper bottle to the lower one only when the animal is expiring air.

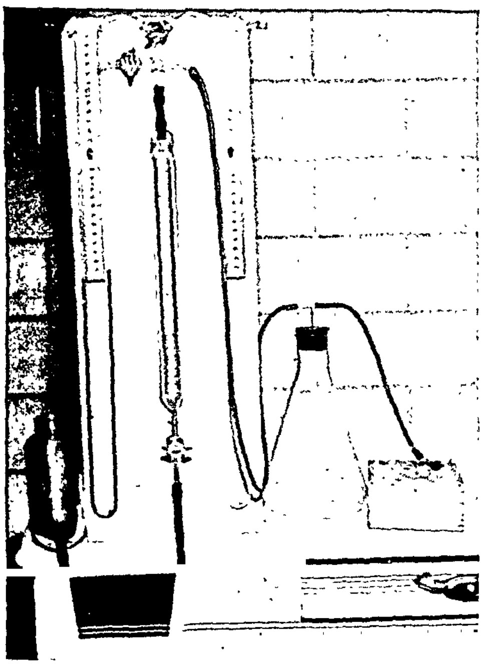

Fig. 1.

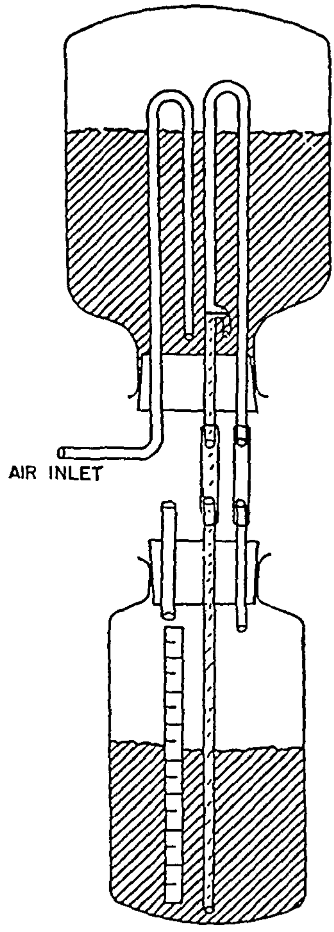

Fig. 2.

Fig. 1. Mercury collecting column for expired air.
Fig. 2. A special water trap for collecting expired air.

For practical purposes, during use of this method, the tube is set so that the animal breathes against approximately ½ cm. of water pressure, this value being negligible as an obstruction to respiration. The respiratory volume is then read directly from the calibration of the lower bottle. The extra tubes shown in the diagram are necessary to prevent airblocks within the apparatus.

Measurements using the head valve method were made only on mice because of the difficulty of eliminating dead space within the mask when larger animals

were used. With mice, practically all of this dead space was eliminated by placing large quantities of vaseline between the mouse's face and the form fitting mask. In the use of the tracheal valve method, the cannula was devised so that the dead space was essentially equal to that of the normal animal. In using this method, the animal was anesthetized with ether, the trachea cannulated, and anesthesia stopped. During the somnolent phase, the animal breathes regularly without struggling and measurements with the tracheal valve were made at this point in the recovery from anesthesia. All measurements were of necessity made with the animal on its back with some restraint necessary to hold it in place.

B. *The oscilloscopic respirograph method.* The respirograph method used in this study for the determination of respiratory volume involves an entirely new principle, and approaches normal physiological conditions more nearly than any method which has been found in the literature. In most methods of determining respiratory volume animals must breathe against valves or there is inertia in moving parts of the apparatus. Nearly all methods utilize masks with considerable dead space, and several require pure oxygen. The respirograph described here, therefore, has been so devised that the animal breathes normal air at all times, the dead space within the mask causes no errors because of constant renewal of air, and the animal breathes against a resistance many times less than that caused by the usual valve.

The respirograph apparatus is illustrated in figure 3. Water flows directly from the upper to the lower bottle. Then, from the lower bottle air flows past the head of the mouse into the upper bottle, thereby completing a closed circuit. The rate of air flow is adjusted so that the volume of air is at least five times as great as that required for normal respiration of the animal. A third tube leads from the headpiece to an airtight bellows on the top of which is an electrical condenser made of alternate layers of insulating paper and tinfoil. As the animal breathes in and out, pressure within the bottles and the bellows alternately increases and decreases by a minute amount. The plates of the condenser likewise alternately become closer and farther apart. By use of a special electrical apparatus, shown in figure 4, variation of the electrical capacity of the condenser causes a wave to rise and fall on the screen of a cathode-ray oscillograph as shown in figure 3. The syringe connected to the bellows is used to calibrate the apparatus with each determination of respiratory volume. All readings may be made directly from the screen of the oscilloscope or the respiratory pattern of the animal's breathing may be accurately recorded with a continuous camera as shown in figure 5. Sensitivity of the apparatus is such that 1/1,000,000 of an atmosphere pressure change can be detected, and 1/100,000 of an atmosphere pressure change can be measured accurately. Errors in these measurements may occur, however, when the connecting tubes are too small, thereby causing a lag in the establishment of pressure equilibria throughout the system. The use of large tubes and frequent testing of the equipment minimize this error.

The electrical apparatus shown in figure 4 operates as follows: The 6J5 tube is simply an audio oscillator operating at approximately 6000 cycles. The output of this feeds into a capacity-resistor bridge circuit which normally is

balanced at its midpoint to near extinction of the oscillator signal. The signal from the midpoint of the bridge is fed into a two stage amplifier utilizing two 6SJ7 tubes. The output of the second stage of amplification is coupled directly to the input of an ordinary commercial oscilloscope, but part of this output signal is abolished by the short-circuiting action of a 6H6 tube which is used in a limiter circuit. This limiter circuit removes a great portion of the background signal and leaves essentially the signal which is modulated. Obviously the voltage of the signal recorded on the oscilloscope depends on how nearly balanced the bridge circuit happens to be. The varying pressure condenser forms a part of this bridge circuit at point *X*. Therefore, when the condenser plates are

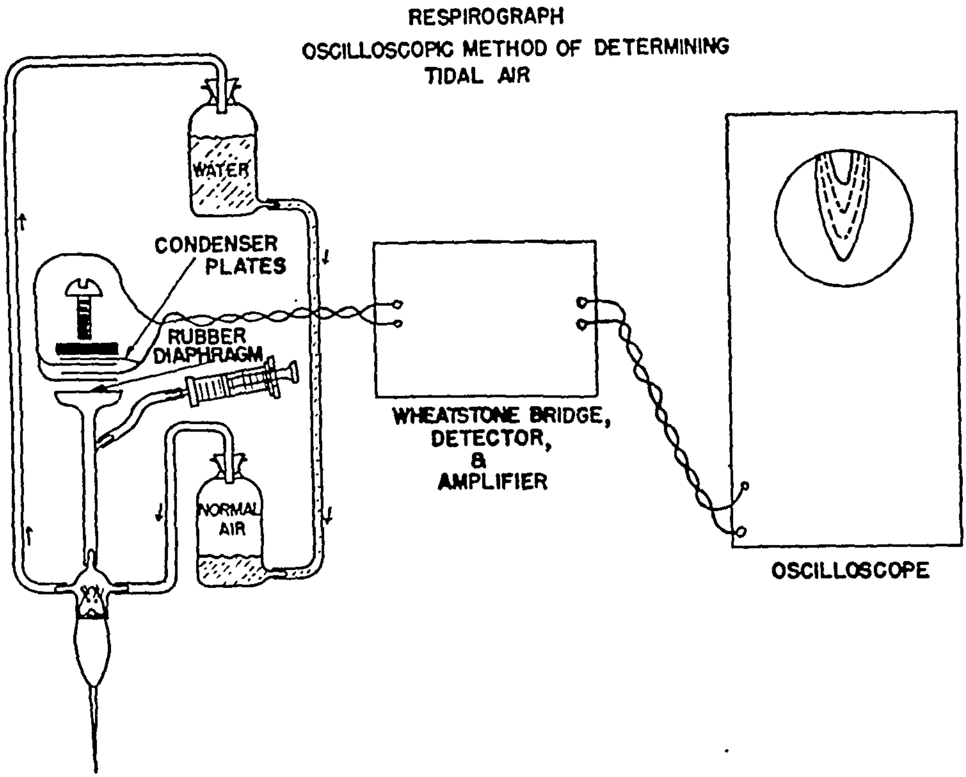

Fig. 3. Diagram of the oscilloscopic respirograph.

pressed closer together or are spread farther apart, the bridge circuit is either more or less nearly balanced, and likewise the quantity of signal which passes through the amplifier varies with the capacity of the pressure condenser. The horizontal sweep of the oscilloscope is synchronized with the oscillator signal to give a rising and falling wave as shown in figure 3. To give a rising and falling line for camera recording, the horizontal sweep is adjusted to zero.

Animals tested by the above modified respirograph method were under as nearly normal physiological conditions as possible except for the matter of fear. This was overcome by allowing the animal to remain in the headpiece for a long period of time before actual measurements were made. As a matter of practice,

after several minutes in the headpiece, the animal remained quietly in place and breathed very evenly, as shown in the respirogram of figure 5. The volume of the bottles used in the experiments was adjusted for different animals so that enough air was present to last usually for 10 minutes, and so that the operating

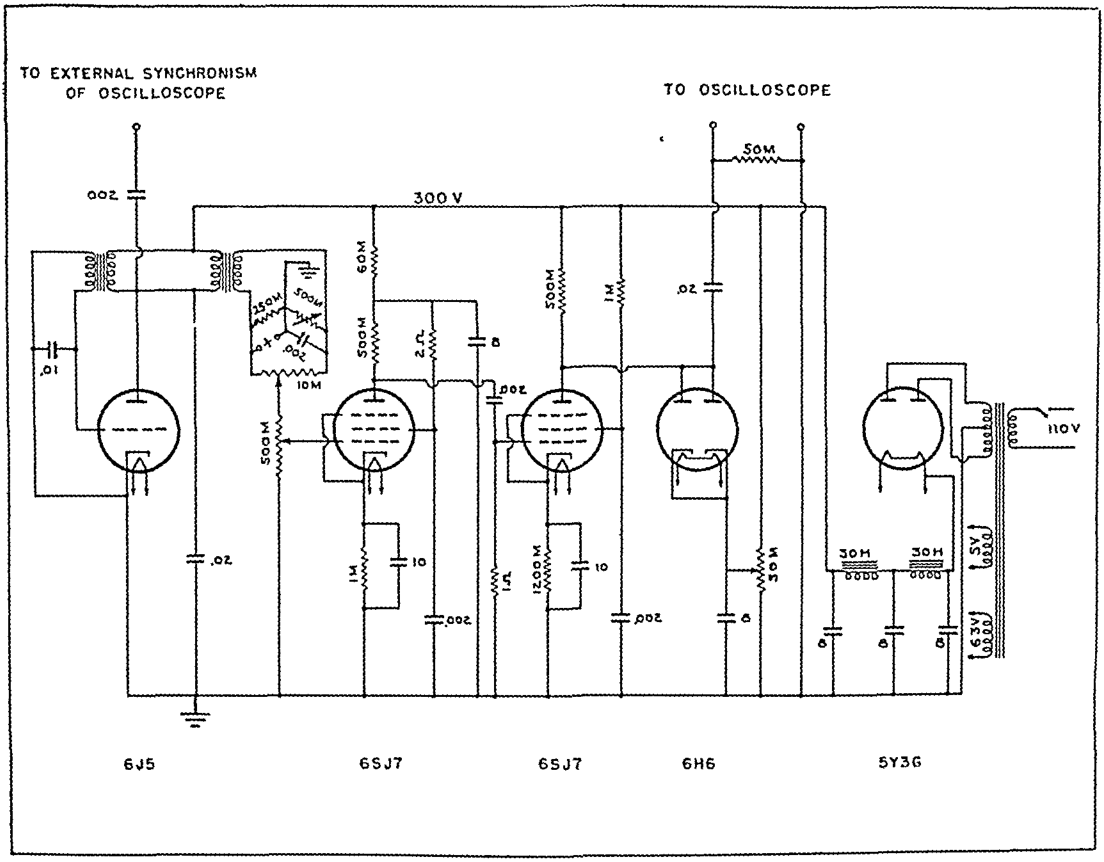

Fig. 4. Electrical diagram of capacity recorder used in the oscilloscopic respirograph.

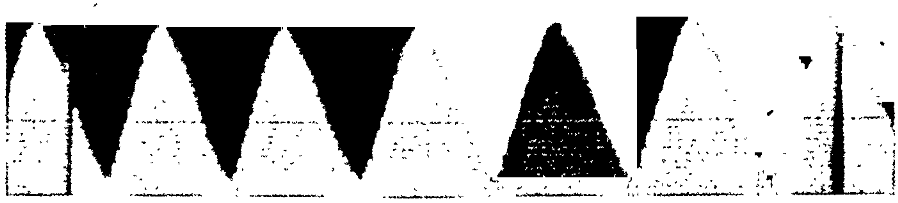

Fig. 5. Respirogram recorded from a guinea pig (time marks every 3 sec.).

pressure within the system varied from atmospheric pressure approximately 1/5000 of an atmosphere with each respiratory cycle. Before making measurements, the apparatus was tested for leaks, and these were eliminated by the use of petrolatum at all joints, including the gap between the head of the animal and the edge of the headpiece.

TABLE 1

	METHOD	NO. DETERM.	WT. (GRAMS)	RESP./MIN.	TIDAL AIR (cc.)	RESP. VOL/MIN. (cc.)
Mice	1	56	Min. 12 Max. 26 Av. 19.8	84 230 163.4	0.09 0.23 0.154	11.1 35.8 24.54
Mice	2	15	Min. 12 Max. 25 Av. 20.7			20.5 35.5 28.1
Mice	3	7	Min. 16 Max. 21 Av. 18.8			11.4 24.0 17.7
Cotton rats	1	27	Min. 49 Max. 130 Av. 76.8	75 115 94.5	0.24 0.70 0.353	22.8 71.4 39.6
Hamsters	1	65	Min. 65 Max. 134 Av. 91.6	33 127 73.6	0.42 1.16 0.826	33.3 82.8 60.9
Hamsters	3	34	Min. 74 Max. 121 Av. 95.7			25 65 47.8
White rats	1	35	Min. 63 Max. 152 Av. 112.8	66 114 85.5	0.60 1.25 0.865	49.8 101.2 72.9
White rats	3	32	Min. 63 Max. 179 Av. 110.2			56 102 75.8
G. P.	1	61	Min. 274 Max. 941 Av. 466	69 104 90.3	1.0 3.9 1.75	100 382.2 155.6
G. P.	3	49	Min. 274 Max. 941 Av. 477			87 329 154.1
Rabbits	4	31	Min. 792 Max. 3090 Av. 2069			279 1208 800
Monkeys	5	6	Min. 2050 Max. 3080 Av. 2682	31 52 40	9.8 29.1 21.2	311 1410 863
Man	5	10	Min. 55,700 Max. 82,100 Av. 68,500	10.5 19.3 14.2	315 745 616.5	4,900 12,200 8,732

Methods: 1. Using oscilloscopic respirograph and recording directly from the screen of the oscilloscope.
2. Using head valve method and collecting expired air over mercury column.
3. Using tracheal valve method and collecting expired air over mercury column.
4. Using tracheal valve method and collecting expired air in automatic water trap.
5. Using oscilloscopic respirograph and recording with a camera on a constantly moving film.

RESULTS. Data obtained in this laboratory are presented in table 1. In general it is felt that the more accurate values have been obtained by the oscilloscopic respirograph method, and the valve methods may be considered as one

means of checking the results. In rabbits, however, panting made it impossible to obtain consistent results by any method as long as the animal was awake. For this reason, only values obtained by the tracheal valve method are reported.

DISCUSSION. The table and values are self explanatory, and there is little necessity to analyze the data. An attempt has been made, however, to find a formula which will correlate respiratory volume and weight of the animal (for any species). In table 2, therefore, is shown the ratio of the respiratory volume to various mathematical functions of the weight. The respiratory volume divided by the animal's weight (column 5) shows a general decline in value as the animal becomes larger. If, on the other hand, one assumes that the respiratory volume follows the body surface of the animal, then the respiratory volume divided by the $\frac{2}{3}$ power of the weight should be approximately constant.

TABLE 2

Comparison of respiratory volume with various functions of the weight

ANIMAL	WT. (GRAMS)	RESP. VOL/MIN (CC.)		RESP. VOL/MIN. (CC.) ÷ WT. (GRAMS)	RESP. VOL/MIN. (CC.) ÷ WT.$^{2/3}$ (GRAMS)	RESP. VOL/MIN. (CC.) ÷ WT.$^{3/4}$ (GRAMS)
		Actual	Calculated			
Mouse	19.8	24.5	19.9	1.24	3.35	2.54
Cotton rat	76.8	39.6	55.5	0.52	2.20	1.52
Hamster	91.6	60.9	62.1	0.67	3.00	2.06
White rat	112.8	72.9	72.9	0.65	3.14	2.10
Guinea pig	466.0	155.6	210.6	0.33	2.60	1.55
Rabbit	2069.0	800.0	634.0	0.39	4.88	2.62
Monkey	2682.0	863.5	785.0	0.32	4.54	2.35
Man	68,500.0	8732.0	8900.0	0.13	5.19	2.06
				0.52	3.61	2.10

This is based on the fact that the surface area of symmetrical bodies is proportional to the $\frac{2}{3}$ power of their weight. Such a calculation with the measured results does not give a constant but instead a slightly rising figure (column 6). This indicates that the respiratory volume is not solely a function of body surface but rather that the mass of the body has some effect on the total respiratory volume. If, therefore, one chooses a function of the weight somewhere between the $\frac{2}{3}$ power and the first power a function should be found which gives a more constant factor. In column 7 the respiratory volume has been divided by the weight to the $\frac{3}{4}$ power. It will be noted that from the smallest to the largest animal, the values remain considerably more constant. From the average of these values the following formula has been derived:

$$\text{Resp. Vol. per Min. in cc.} = 2.10 \times (\text{Wt. in Grams})^{3/4}$$

Using this formula, the average respiratory volumes of the animals which have been tested were calculated and are shown in column 4 of table 2 for comparison with measured values (column 3). Obviously, other functions of the weight such as 0.73, 0.74, or 0.76 power of the weight probably would give even

better correlation between weight and respiratory volume. The data, however, even though derived from the study of 428 animals, are insufficient to establish any one of these values as the absolutely correct one. Therefore, the $\frac{3}{4}$ power of the weight has been chosen for the sake of simplicity. This has been found to give a relatively satisfactory correlation between weight and respiratory volume. It is interesting that Kleiber (3) found oxygen consumption in animals, from the rat to the steer, to vary also with the $\frac{3}{4}$ power of the weight.

It has been of interest to note the reactions of animals immediately after being placed in the respirometer. There have been many statements to the effect that animals under conditions of fear and noxious stimuli will hold their breath. In one experiment while anesthetizing a monkey with ether, records taken during the actual anesthetization showed that breathing became irregular in association with struggling, but there was no interruption of breathing. Occasionally animals appeared to hold their breath when they were first put into the respirometer, but actual records invariably showed that there were rapid though shallow movements of air into and out of the lungs.

SUMMARY

1. Several methods of determining the respiratory volume, including the oscilloscopic respirograph, have been presented.

2. Data on quiet respiration in 428 animals of 8 species have been presented.

3. The data presented indicate that the respiratory volume varies directly neither with the weight of the body nor with the body surface but approximately with the $\frac{3}{4}$ power of the weight.

4. A formula has been derived for the determination of respiratory volume of an animal provided the weight is known.

Grateful acknowledgment is made to Dr. Theodor Rosebury for his suggestion of this problem and to Cpl. Dellas A. Cosby for his assistance in carrying out many of the routine measurements.

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ANALYSIS OF RESPIRATORY PATTERNS IN LABORATORY ANIMALS[1]

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Very scanty information is available in the literature on methods for recording the respiratory pattern of smaller animals. Loosli et al. (3) developed a method for making such a record from the mouse, but this method required that the animal breathe pure oxygen. This method was not applied to animals other than the mouse.

An oscilloscopic method of recording the respirogram has been developed in this laboratory, and the study of the respiratory volumes of several hundred laboratory animals by this method has been reported (2). Most of these original records were examined directly from the oscilloscopic screen, a procedure which was adequate for the study of respiratory volume. Over a hundred permanent records have subsequently been made on photographic film. From these latter records and from data obtained in the previous study (2), an extensive analysis of the different aspects of the respiratory pattern has been made.

RESULTS. Normal respiratory patterns for eight animals of different species are shown in figure 1. The actual patterns of respiration are obvious from the pictures, but other considerations not immediately apparent have been noted from a mathematical study of these patterns.

a. *Comparison of the respiratory patterns from different animals.* From the mouse to man, there is considerable uniformity of the respiratory pattern, as may be seen from the records. An exception to this has been that the ratio of inspiration to expiration has varied from 0.42 to 2.17 if we consider inspiration to begin when the lungs are $\frac{1}{2}$ filled and to end when they are $\frac{1}{2}$ emptied during any one cycle. This ratio has varied, however, within all species, and there is no constant trend from small animals to larger animals.

In general, smaller animals tend to have more uniformity of the respiratory cycle. To illustrate this point, the eight respiratory patterns shown for guinea pigs in figure 2 were chosen at random. With the exception of the last pattern, and making allowance for rate and volume of respiration, the records can be almost superimposed upon each other.

b. *Tidal air.* Tidal air has been found to vary, approximately, directly with the weight of the animal as can be ascertained from values in table 1 of the previous paper (2). From an average of 300 records of normal quiet breathing, the following formula has been derived:

$$\text{Tidal Air (cc.)} = 0.0074\ \text{Weight (in grams)} \qquad (1)$$

[1] Work carried out at Camp Detrick, Frederick, Maryland, from March, 1945 to September, 1945.

[2] Present address: Warm Springs, Ga.

Using this formula for the calculation of tidal airs of the various species of animals studied, it was found that the average deviation of the calculated values from the measured values was ±21.5 per cent.

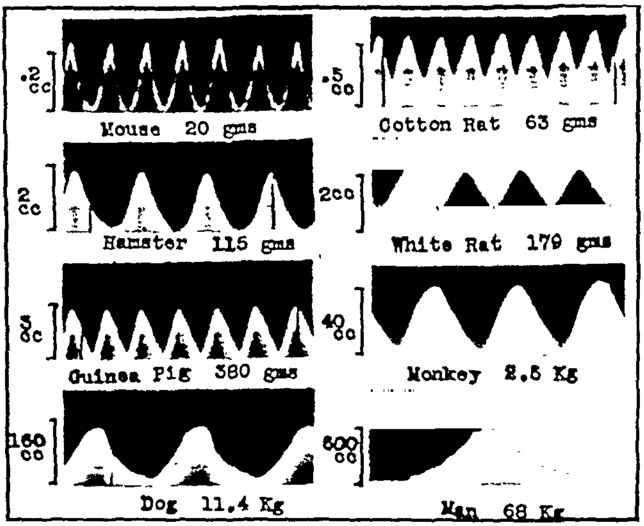

Fig. 1. Normal respiratory patterns from different animals. (Inspiration up, expiration down. Time marks—3 sec.)

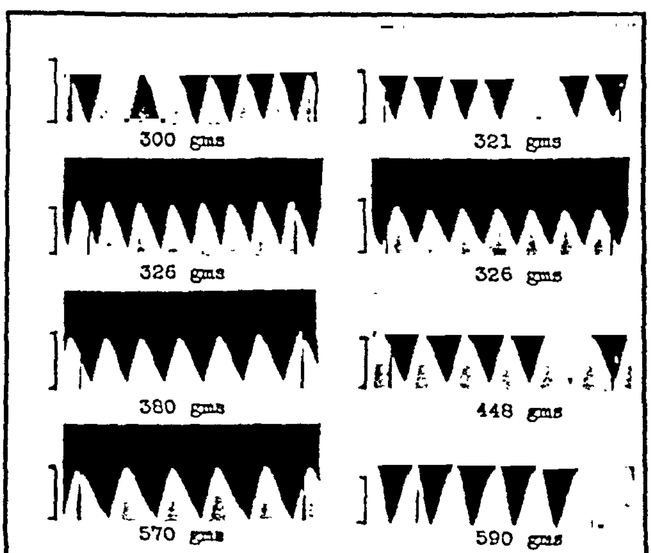

Fig. 2. Normal respiratory patterns of guinea pigs chosen at random. (Inspiration up, expiration down. Calibration—3 cc. Time marks—3 sec.)

c. *Rate of breathing.* As shown in the previous paper (2), the minute respiratory volume is proportional approximately to the $\frac{3}{4}$ power of the weight. Since the tidal air is proportional to weight, and,

$$\text{Tidal Air} \times \text{Rate} = \text{Minute Respiratory Volume},$$

one may derive the formula for rate of breathing:

$$\text{Rate of Breathing (per minute)} = \frac{295}{\text{Weight (in grams)}^{\frac{1}{4}}} \tag{2}$$

Testing this formula with the measured breathing rate of approximately 300 animals of various species, the calculated values deviated from the measured values an average of ± 17 per cent. This deviation was due mainly to the fact that mice and guinea pigs breathe somewhat faster than would be calculated by the formula. The data for tidal air and rate of breathing on which the above calculations are based are presented in the preceding paper (2).

d. *Rate of air flow on inspiration and expiration.* If we assume that the respiratory patterns of all species are relatively symmetrical, and remembering that the minute respiratory volume varies with the $\frac{3}{4}$ power of the weight, it follows that inspiratory rate and expiratory rate at any given point in the cycle vary approximately with the $\frac{3}{4}$ power of the animal's weight.

For the midpoint in the inspiratory curves and the midpoint in the expiratory curve of 100 photographic records, the following formulae have been derived from the average slope of the curves:

$$\text{Inspiration (in cc./sec.)} = 0.15\ W^{3/4}\ \text{(in grams)} \tag{3}$$

$$\text{Expiration (in cc./sec.)} = 0.15\ W^{3/4}\ \text{(in grams)} \tag{4}$$

In records from mice, cotton rats, white rats, hamsters, guinea pigs, monkeys, rabbits, and men, the values calculated from the inspiratory formula deviated an average of ± 16.7 per cent from the measured values, and values calculated from the expiratory formula deviated an average of 21.8 per cent from the measured values.

e. *Speed of air flow on inspiration and expiration.* The dimensions of homogeneous symmetrical bodies vary directly with the $\frac{1}{3}$ power of the weight. By measurement of the tracheae of ten mice, giving an average of 1.0 mm. internal diameter, and assuming man's trachea to be 15 mm. internal diameter, it has been calculated that the diameter of the trachea obeys almost precisely this $\frac{1}{3}$ power law. It follows mathematically that the cross-sectional areas of the tracheae vary with the $\frac{2}{3}$ power of the weight. Because the inspiratory rate and the expiratory rate of the various animals vary approximately with the $\frac{3}{4}$ power of the weight as shown above (this is nearly equal to the $\frac{2}{3}$ power of weight), there is approximately a proportional change between the increase in cross-sectional area of the trachea and the quantity of air flowing. Therefore, the linear speed at which air flows through the tracheae of animals of different species is approximately the same at each point in the respiratory cycle for all species. As a test of this premise, using the measured internal diameter of 1 mm. for the mouse's trachea and the arbitrary internal diameter of 15 mm. for the human trachea, the inspiratory speed of air for ten measurements in the mouse as calculated from the slope of the respiratory pattern was 2.05 meters/sec. compared with 2.95 meters/sec. for ten measurements in man. The expiratory speed in the mouse was 2.65 meters/sec. compared with 2.8 meters/sec. in man.

f. *Pressure differential in the trachea.* Pressure drop through a tube is proportional to the *length* of the tube and inversely proportional to the *square of the radius* of the tube provided the speed of flow remains the same. Since both the length and the radius of the trachea are proportional to the $\frac{1}{3}$ power of the weight of different animals, and because the speed of air flowing through the tracheae of different animals remains approximately constant at any one point of the cycle, the following formula may be derived for pressure drop in the trachea:

$$\text{Pressure drop} = \frac{\text{Constant}}{\text{Weight}^{\frac{1}{3}}} \tag{5}$$

The value of this constant cannot be determined by present methods of measurement because of difficulty in measuring instantaneous pressure in the recesses of the lung.

It follows from the formula, however, that even though the mouse's trachea is 15 times shorter than man's trachea, the total pressure drop from the atmosphere to the bifurcation of the trachea is 15 times as great in the mouse as in man. This is a startling calculation when considered without further thought, but in man the trachea must branch a considerably greater number of times than in the mouse before it reaches the terminal bronchioles. It is in these branches that a great pressure drop is found in man and particularly so in asthmatics. Perhaps if we could calculate or measure pressure gradients throughout the lung, we would find the total pressure change from the atmosphere to the alveoli to be approximately the same in the mouse as in man.

g. *The nose as a particle precipitator.* If we consider the nose to be a settling chamber of the cyclone type, we may apply the formulae for calculating retentive power of the chamber. Retention is proportional to the length of the chamber, inversely proportional to the diameter of the chamber, and inversely proportional to the radius of the various curves within the chamber (1). Making the assumption that the nasal chambers of different species of animals are reasonably symmetrical, we may again apply the law for determining relative dimension, i.e., dimension is proportional to the $\frac{1}{3}$ power of the weight. Because length of the chamber, diameter, and radius of curvature are all proportional to the $\frac{1}{3}$ power of the weight, and because retention is directly proportional to the first of these dimensions and inversely proportional to the last two, it is found that retention of particles in the nasal chambers should be inversely proportional to the $\frac{1}{3}$ power of the weight, or:

$$\text{Retentivity} = \frac{\text{Constant}}{\text{Weight}^{\frac{1}{3}}} \tag{6}$$

Here again the constant cannot be determined because of the impossibility of making the necessary measurements. From this relationship, it follows that in order to be precipitated in the nose by settling, a particle must have a terminal settling velocity 15 times as great in man as in the mouse. From Stokes' law, one finds that the terminal velocity of settling for homogeneous particles is proportional to the square of the diameter of the particle (1). In other words, a

2 micron particle would settle out in the nasal chamber of the mouse as readily as would a 7.8 micron particle in man. It appears, therefore, that the filtering efficiency of the mouse's nose should be considerably greater than the efficiency of the human nose. The above calculations, of course, do not include the effect of cilia and hairs which add greatly to the efficiency of the nasal chamber as a filter. Also, the objection may be raised that the human and mouse noses are not symmetrical. This is a valid objection but only partly so, for the asymmetry of

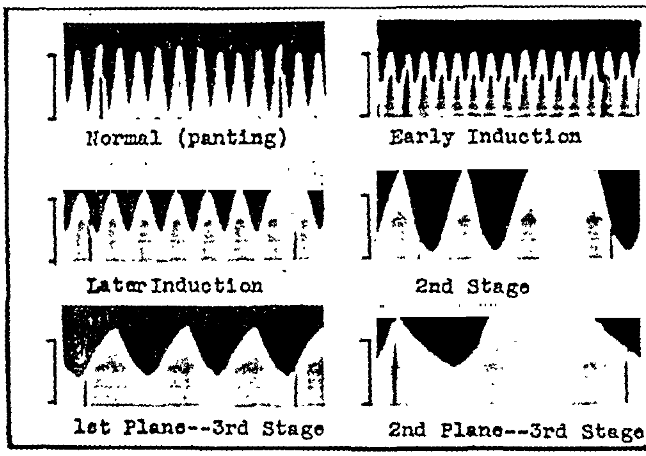

Fig. 3. Respiratory patterns from a rabbit in different stages of nembutal anesthesia. (Inspiration up, expiration down. Calibration—20 cc. Time marks—3 sec.)

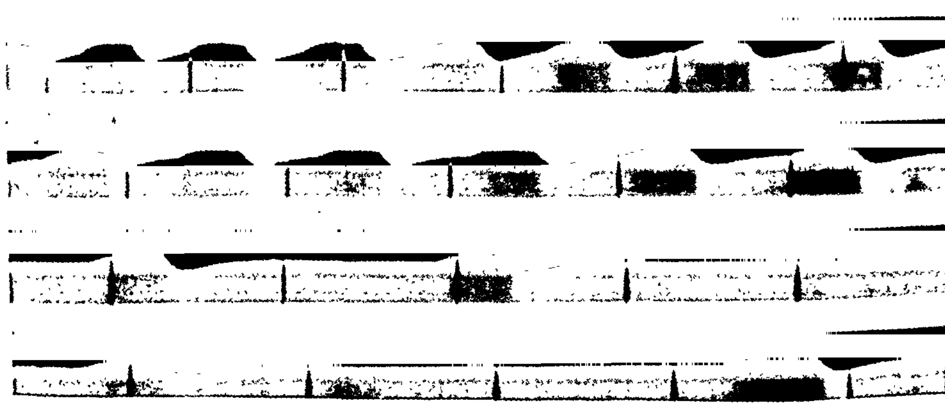

Fig. 4. Respiratory pattern from a rabbit dying under nembutal anesthesia. (Inspiration up, expiration down. Time marks—3 sec.)

any one dimension must be at least 15 fold in order to equalize the two nasal chambers in efficiency. Obviously, such asymmetry does not exist.

h. *Illustrative records of changing respiratory patterns.* Figures 3 and 4 illustrate the method of studying changing respiratory patterns, due in this case to nembutal anesthesia. The records in figure 3 were made during the course of ½ hour after the administration of 0.13 gram of nembutal intraperitoneally to a 2 kgm. rabbit. A record of breathing was obtained through all stages of anesthesia down to the 2nd plane of the 3rd stage at which point the animal's narcosis came to equilibrium. The first effect of nembutal on the panting rabbit was to

decrease the tidal air without decreasing the rate. Later, the rate of breathing also decreased, and this was followed by further slowing of breathing and a secondary increase in tidal air during the 2nd stage. From the second stage through the 1st plane of the 3rd stage, there was little change in the rate but considerable decrease in tidal air. Once surgical anesthesia had been reached, the shape of the respiratory pattern began to change with the appearance of irregularities in the inspiratory and expiratory slopes. Also, as deeper anesthesia was attained, the rate decreased, but the tidal air remained fairly constant. In these records it is seen that there are many changes in respiration during anesthesia which cannot be discerned by simple observation of the subject.

The respiratory pattern shown in figure 4 is that of a rabbit which received 0.3 gram of nembutal, passed through all stages of anesthesia, and died in approximately 5 minutes. The portion of the record shown in the figure is that immediately preceding death, including the last breath. The record shows that there was a markedly increasing delay between inspirations as death approached, but at the same time there was little decrease in the volume inspired with each succeeding breath. It would appear, therefore, that the cause of death was an increasing delay between phrenic nerve volleys until asphyxia occurred. The actual strength of each nerve volley was apparently almost as strong, if not just as strong, during the last inspiration as during earlier ones.

These two experiments on anesthesia with nembutal have been presented not as an explanation of the action of nembutal but to illustrate the possibilities of new knowledge which may be gained from the study of respirograms. This work may be extended greatly to include aerosols, vapors, and other drugs of all nature which may affect the respiration.

SUMMARY

1. Respiratory patterns from mice, cotton rats, hamsters, white rats, guinea pigs, rabbits, monkeys, dogs, and men have been illustrated and analyzed.

2. In general, the respiratory pattern varies little between one species of animals and another except for rate and tidal air.

3. Within any one species of the smaller animals, the respiratory pattern is relatively constant.

4. Formulae based on weight have been derived for tidal air, rate of breathing, inspiratory and expiratory rate of flow, inspiratory and expiratory speed of flow, pressure drop in the trachea, and retention of particles within the nasal passages. In general, the calculated values from these formulae agreed within 20 per cent of those which could be measured. Considering the great number of variables in respiratory dynamics, this agreement is considered to be reasonable.

5. As an example of information which can be gained from respiratory patterns, two experiments on the anesthetization of rabbits with nembutal are presented.

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PITUITARY AND OVARIAN DYSFUNCTION IN EXPERIMENTAL DIABETES

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Clinical investigators have observed ovarian insufficiency in diabetic patients (1, 2), and subnormal development of the gonads in experimental diabetes has been noted (3, 4, 5). Experiments with parabiotic rats have indicated that the gonadal deficiency may be due to decreased gonadotrophin secretion from the pituitary (5).

The investigation reported in this paper was undertaken to examine further the pituitary gonadotrophic function in diabetic rats, and the response of the reproductive tract of diabetic rats to exogenous gonadotrophins and estrogens.

Materials and methods. The investigations were made using immature or adult female albino rats of a Wistar strain obtained from Blaine's, Media, Pa. The rats were given Rockland's diet and water *ad libitum*.

Diabetes was produced by means of intravenous administration of 50 to 60 mgm./kgm. of alloxan in 5 per cent solution. Blood glucose determinations were made using the method of Reinecke (6) adapted to the Evelyn colorimeter. The blood samples were obtained from the cut tip of the tail, and determinations were made every second or third day during the course of the experiment. No insulin was given.

The crude anterior pituitary extract (APE) was an alkaline saline extract of fresh frozen beef pituitary mixed in a Waring blendor and filtered through a sintered glass filter. The APE was administered by subcutaneous injection once each day in 250 mgm. equivalents (1 cc. of APE) during the experimental period. Autopsy was performed on the day following the last injection.

The purified FSH[1] preparations were given in 500 mgm. equivalents (total dose) in 9 injections over $4\frac{1}{2}$ days, with autopsy on the fifth day.

Unilateral ovariectomy in adult rats was performed under ether anesthesia and the right ovary was removed in all cases. The ovaries were weighed immediately after removal and the average weight was compared with the average for the remaining (left) ovaries at the time of autopsy, 10 days later. Bilateral ovariectomy was performed on adult rats, which were then used to determine the response of the uterus of diabetic and control rats to estrogen administration. Alloxan injections were made in the unilaterally and bilaterally ovariectomized rats at the time of ovariectomy.

Estrone, when used, was given in corn oil and was injected once daily for 3 days; autopsy was performed on the fourth day. Unilateral adrenalectomy and adrenal enucleation were performed under ether anesthesia using a dorsal ap-

[1] Kindly supplied by Dr. R. K. Meyer and Dr. H. M. McShan, University of Wisconsin.

proach. After adrenal enucleation the rats were given 1 per cent saline as drinking water for a period of 10 days.

RESULTS AND DISCUSSION. Right unilateral ovariectomy was performed on female rats 41 days of age to determine whether the remaining ovary of a diabetic unilateral castrate rat would be capable of hypertrophy to the extent of that of a non-diabetic animal. Fourteen rats were kept as controls and 18 rats were given 50–60 mgm. of alloxan/kgm. at the time of unilateral ovariectomy, so that diabetes ensued within 24 hours after the ovary was removed. Table 1 includes the average body weight and the average weight of the removed ovaries in both the control and the experimental animals before alloxan treatment. Random selection determined the animals for each group, but the control group weights at the beginning of the experiment were slightly greater than those of the experimental group.

No further treatment was given and the blood glucose determinations were made in both groups every second or third day for the purpose of following the course of diabetes. The over-all average blood sugar values for control rats was 143 mgm./100 cc. (not fasting) and the average for the diabetic rats was 462 mgm./100 cc. (table 1). Ten days after unilateral ovariectomy the rats were autopsied. The non-diabetic rat ovaries were hypertrophied to the extent that the average weights were 26.4 mgm. compared to 10.8 mgm. for the diabetic group. The average weights of the right ovaries in the two groups were 10.7 and 8.5 mgm. The figures represent gains of 147 per cent and 25 per cent respectively over the right ovarian weights for the two groups at the beginning of the experiment.

The fourteen ovaries removed from the non-diabetic rats at the time of autopsy contained several to numerous corpora lutea. Four of 18 ovaries from the diabetic rats contained corpora lutea and 14 of 18 contained only follicles. The histological preparations confirmed the gross examination and showed that ovaries of 14 of the diabetic rats consisted largely of small and medium follicles with signs of atresia in some. Although 4 of 18 diabetic rat ovaries contained corpora lutea, these four ovaries were smaller, and had fewer corpora lutea and smaller follicles than were found in the ovaries of the control rats. The average uterine weights of the diabetic rats were approximately one-half as great as the control rat uterine weights, and the pituitary glands were approximately one-third smaller than the control rat pituitary glands.

A second group of rats of the same age was subjected to the same experimental procedure as the preceding group to determine the reproducibility of the results. Five non-diabetics and six diabetic rats were used; the results are included in table 1. The results were in accord with the results obtained with the first group. Marked hypertrophy of the left ovary occurred in non-diabetic rats (91 per cent increase) while only a slight increase was found in the diabetic rats (36 per cent). Non-diabetic uterine weights were likewise much greater (107 per cent to 246 per cent) than were corresponding organ weights in the diabetic rats.

Changes in weight of the pituitary gland also were striking. The control rat pituitary gland weights were 46 per cent and 63 per cent greater than those of

diabetic rats in corresponding groups. Statistical analysis[2] of the data on ovarian and pituitary weights in table 1 indicated the difference in the means was highly significant since p = <0.01 for both ovarian and pituitary weights. The decreased ovarian, uterine, and pituitary weights suggested decreased production of gonadotrophin in the diabetic animals. The possibility existed, however, that the smaller ovaries and uteri were due to the failure of the end organs to respond to secreted gonadotrophins.

As a test of this suggestion, exogenous gonadotrophins were administered to determine whether the ovaries of diabetic animals would respond to gonadotrophins.

TABLE 1

Ovarian hypertrophy in diabetic and non-diabetic rats after unilateral ovariectomy

BEFORE TREATMENT			AFTER TREATMENT WITH ALLOXAN						
No. of rats	Bd. wt.	Right ovary wt.	Average blood glucose	Bd. wt.	Left ovary wt.	% increase	Ovarian response	Uterine weight	Pit. wt.
	gm.	*mgm.*	*mgm. per 100 cc.*	*gm.*	*mgm.*			*mgm.*	*mgm.*
14	97	10.7	143	110	26.4	147	Corpora lutea	172.0	7.0
18	90	8.5	462	86	10.8	25	Corpora lutea (4) Follicles (14)	83.4	4.8
5	94	12.0	133	112	22.8	91	Corpora lutea (3) Follicles (2)	173.0	7.5
6	90	10.0	421	83	13.6	36	Corpora lutea (1) Follicles (5)	50.4	4.6

The first gonadotrophin used was a crude saline extract of beef anterior pituitary glands (APE), which was injected at a level of 1 cc. per day for 10 days into young female rats that were 49–50 days of age. The animals were divided by random selection into four groups, as follows: *a*, non-diabetic rats; *b*, non-diabetic rats given APE; *c*, diabetic rats, and *d*, diabetic rats given APE. After 10 days it was found (table 2) that the ovaries of the diabetic rats were smaller than those of the non-diabetic groups both with and without APE treatment; the blood glucose determinations revealed that the two diabetic groups had similar degrees of diabetes. Examination of the data discloses that APE increased ovarian weights from 31.9 to 48.6 (52 per cent) in non-diabetic and from 25.4 to 37.6 mgm. (48 per cent) in diabetic rats. Statistical analysis of the data yields p = 0.05, and p = 0.01 for non-diabetic and diabetic APE-treated rat ovaries, re-

[2] Statistical analyses were obtained using the formulae: S.E. = $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$ $T = \frac{M_1 - M_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}$, and the probability table of Fischer.

spectively. The gonadotrophic potency of the preparation was apparently low, yet the results are significant when analyzed statistically. The adrenal increases were statistically significant in the non-diabetic group and highly significant in the diabetic APE-treated group. The untreated *diabetic* rat ovaries were 20 per cent smaller than untreated *non-diabetic* rat ovaries, and the weights of the *diabetic* APE-treated rat ovaries were 22.6 per cent less than those of the *non-diabetic* APE-treated ovaries. Thus the APE treatment produced comparable degrees of hypertrophy in diabetic and in non-diabetic animals, and the diabetic animals had smaller ovaries than non-diabetic ones in both untreated and APE-treated groups. The absolute differences between the two groups can be attributed to differences in the endogenous gonadotrophin from the pituitary gland. The results nevertheless suggested that the smaller gonads found in diabetic animals might have been a result of the faulty nutrition of diabetic animals, with consequent inability to utilize gonadotrophin secretion. To test this possibility, animals of similar age and weight to those of the previous experiment were fasted for a period of five days. Six of the rats were given no other treatment and 5 rats

TABLE 2

Organ response to crude APE in normal and diabetic rats

NO. OF ANIMAL	TREATMENT	BD. WT.	AVERAGE BLOOD GLUCOSE	OVARIAN WT.	% OVARIAN WT. INCREASE	UTERINE WT.	ADR. WT.	PIT. WT.
		gm.	*mgm/100 cc.*	*mgm.*		*mgm.*	*mgm.*	*mgm.*
5	Controls	114	165	31.9		189.0	36.6	6.4
5	APE	119	162	48.6	52	98.0	48.2	6.0
10	Diabetic	105	495	25.4		75.7	36.8	5.5
9	Diabetic + APE	115	483	37.6	48	105.7	54.3	5.1

were given APE in 250 mgm. equivalents for the 5-day period. The rats then were sacrificed, and weights of ovaries, adrenals, and pituitary glands were obtained. In the 5-day period of fasting there was a 17 per cent increase in the size of the ovaries of the first APE-treated group, as compared with those of the control fasted rats, thus indicating that starving animals still can respond to gonadotrophin (table 3). In a repetition of the experiment only two of the APE-treated rats survived, yet the ovarian weight increases in the fasted rats given APE were 84.3 per cent, and even this small group gave significant differences statistically. The APE used in the first group of fasted rats was found to have a low order of gonadotrophic potency even when administered to non-fasting animals. Highly significant differences were found in adrenal weights when analyzed statistically. Earlier results indicated (5) that decreased food intakes sufficient to produce body weight losses equal to those of the diabetic rats did not produce ovarian atrophy to the extent seen in diabetic rats. The results with fasted rats supported the thesis that the gonadal deficiency found in diabetes was primarily a result of decreased pituitary function. The pituitary dysfunction in turn was believed to be a result of insulin deficiency and/or the

high blood glucose *per se* of diabetes. The food intake of alloxan diabetic rats was high; in long standing untreated diabetes a marked splanchnomegaly occurred.

To examine further the effects of exogenous pituitary gonadotrophin preparations upon the ovaries of diabetic animals, an experiment was performed with immature 20–22 day female rats in which there is little endogenous pituitary gonadotrophin secretion. Some of the rats were given 60 mgm. of alloxan/kgm. at 20–22 days of age. Blood glucose determinations were made to determine the severity of diabetes; four days later the diabetic rats were divided into two groups so that the rats of each goup had similar degrees of diabetes. One of these groups received FSH treatment, the other was untreated. Purified FSH preparations in 500 mgm. eq. were injected twice daily over $4\frac{1}{2}$ days, with autopsy on the morning of the fifth day. Non-diabetic rats of the same age were used as untreated

TABLE 3

Effects of APE in fasted rats

NO. OF ANIMALS	TREATMENT	BD. WT. AFTER 24 HR. FAST	BD. WT. AFTER 5 DAYS FAST	BLOOD GLUCOSE MGM./100 CC.		OVARIAN WT.	MGM. OVARY PER 100 GM. BD. WT.	ADR.	PIT.
				24 hrs.	120 hrs.				
						mgm.		*mgm.*	*mgm.*
6	Fasted	124	101	121	125	26.0 (12–33)	25.7	38.2	5.1
5	Fasted + APE	119	91	112	120	30.5 (21–46)	33.5	53.4	5.4
4	Fasted	117	97			36.3 (34–38)	37.4	47.7	7.0
2	Fasted + APE	133	120			66.9 (56–78)	55.8	65.9	7.4

and FSH-treated controls. All groups were autopsied on the 6th day after treatment with FSH was started. It will be noted (table 4) that the ovarian response of the immature diabetic rats to exogenous gonadotrophin was in every case greater than that of the corresponding non-diabetic groups that received equivalent doses of the same pituitary preparation. Statistical analyses of the diabetic rat ovarian weights in response to FSH 333, FSH 124, and FSH 130 indicate the results are highly significant ($p =$ less than 0.01). In the non-diabetic groups the response to FSH 130 was highly significant ($p = <0.01$) but the other results were not significant. Thus the diabetic rat ovarian response to exogenous gonadotrophin was equal to or greater than that in the non-diabetic groups of rats. The evidence from this experiment further supports the theory of deficient gonadotrophin secretion in the diabetic animal, since the atrophic ovaries seen in untreated diabetic rats grew and matured if gonadotrophin was administered.

The clinical evidence (2, 7) had indicated subnormal gonadal function in

"controlled" diabetes and the need for hormonal treatment during pregnancy. Previous investigators have reported the subnormal size of gonads in experimental diabetes (3, 5) but no explanation has been given for the cause of this subnormal gonadal function. Changes in the pituitary cytology of diabetic dogs were noted by Kan (4), who observed decreases in the acidophilic cells. Kraus (8) observed decreased number and size of acidophils, increased "Hauptzellen" (chromophobes) and varying changes in the basophils, including "hydropic degeneration". Thomas and Emerson (9) reported marked degranulation of the basophilic cells of the *pars anterior* in diabetic rabbits with no change in the cells of the *pars intermedia*.

TABLE 4

The effects of FSH upon the ovaries and uteri of diabetic and non-diabetic immature rats

NO. OF RATS	TREATMENT	BD. WT.	AV. BOOD GLUCOSE	OV. WT.	% INCREASE OVER CONTROLS	UT. WT.	PIT. WT.
		gm.	*mgm./100 cc.*	*mgm.*		*mgm.*	*mgm.*
8	Non-diabetic	65	131	10.0		34.2	3.6
5	Non-diabetic FSH 333	66	141	16.2	60	28.4	3.0
2	Non-diabetic FSH 124	58	132	13.1	30	28.4	2.9
5	Non-diabetic	63	144	12.7		35.2	3.6
7	Non-diabetic FSH 130	65	165	20.7	63	40.7	3 4
9	Diabetic	54	519	10.6		25.9	2.7
5	Diabetic FSH 333	53	560	19.0	79	27.4	2.8
4	Diabetic FSH 124	53	509	20.2	91	29.9	3.1
5	Diabetic	48	486	9.3		23.0	2.6
10	Diabetic FSH 130	56	398	23.5	153	47.4	2.5

The small uterine weights found in diabetic rats raised the question of the ability of diabetic rats to utilize estrogen. To determine the degree of response of diabetic rats to estrogens, the following experiment was performed using immature 20–21 day ovariectomized female rats given diabetogenic doses of alloxan (50–60 mgm./kgm. intravenously) at the time of ovariectomy. Estrone dosage of 0.5, 1.0 and 1.5γ was begun on the third day following alloxan administration. The estrone was dissolved in corn oil and was given subcutaneously, once each day for 3 days. On the morning of the fourth day the rats were autopsied and the uteri were weighed. Control rats were of the same age and were ovariectomized and given the same estrogen treatment as was given to the diabetic ovariectomized rats. From the data (table 5) it can be noted that the uterine weight response of the diabetic rats was of the same order as that of the non-diabetic ovariectomized rats, indicating utilization of estrogens by the diabetic animal equal to that in non-diabetic animals. Statistical treatment of the data showed highly significant differences ($p = <0.01$) in estrone-treated diabetic and non-diabetic rats, as compared to their controls, and no significant differences in the response of diabetic and non-diabetic groups to equivalent doses of estrogen.

Adult female rats were used to determine the effects of estrone upon the vagi-

nal smear of diabetic and non-diabetic ovariectomized animals. The rats were ovariectomized 7–10 days before the injection of alloxan. After diabetes was established (2–3 days) in the experimental animals, administration of 1γ of estrone in 0.1 cc. corn oil per day was begun. Examination of vaginal smears in both diabetic and non-diabetic groups was made over a period of 18 days. Estrogen was given for 14 days. The record of vaginal cornification can be seen in figure 1, from which it will be apparent that the response of diabetic rats was more regular and slightly greater than that of non-diabetic ovariectomized rats given the same dose. Thus it can be seen that diabetic rats responded to exogenous estrogen in both uterine weight and vaginal cornification tests to an extent equal to or greater than the response of non-diabetic rats.

TABLE 5

Effects of estrone in ovariectomized diabetic and non-diabetic rats

NO. OF RATS	BLOOD GLUCOSE	BD. WT.	TREATMENT	UT. WT.	PIT. WT.
9	Normal	56		26.0	3.3*
6	366	53		28.9	3.2
7	Normal	57	0.5 γ estrone	56.2	3.1
7	553	64	0.5 γ estrone	61.0	3.7
9	Normal	67	1.0 γ estrone	64.1	3.8†
6	388	52	1.0 γ estrone	65.0	3.6
3	Normal	56	1.5 γ estrone	82.5	3.7
5	312	56	1.5 γ estrone	77.4	3.5

* Average weight of 5 pituitaries.

† Average weight of 3 pituitaries.

Since the vaginal smear response to exogenous estrogen appeared to be slightly greater in diabetic than in non-diabetic rats, it seemed important to determine whether the atrophic ovaries of diabetic rats were capable of supporting a normal estrous cycle. A group of nine normal adult female rats were used. Vaginal smears were followed for eleven days preceding alloxan administration to ascertain that cycles were within the normal range. Alloxan injections in diabetogenic doses (50–60 mgm./kgm.) were made intravenously, and the vaginal smears were followed for an additional 25 days following the onset of diabetes (fig. 2). Four of 9 rats were found to have single estrous cycles after diabetes was precipitated, then became acyclic. Five of 9 rats were acyclic for the entire diabetic period. It was of interest to note that rat 11 had an estrous smear beginning the third day of diabetes and lasting for 3 days.

The results indicate that the ovaries of diabetic rats do not function normally, but that the accessory organs are reactive to the estrogen administration with equal or greater sensitivity than are the same organs of normal rats.

It is well known that unilateral adrenalectomy results in hypertrophy of the intact adrenal. The hypertrophy is analogous to ovarian hypertrophy after unilateral ovariectomy, and is dependent upon the secretion of adrenotrophic hormone by the pituitary gland (10). The extent to which adrenal hypertrophy

would occur in diabetic rats appeared to be relevant to the question of whether diabetes resulted in general inactivity of the pituitary gland or if the gonado-

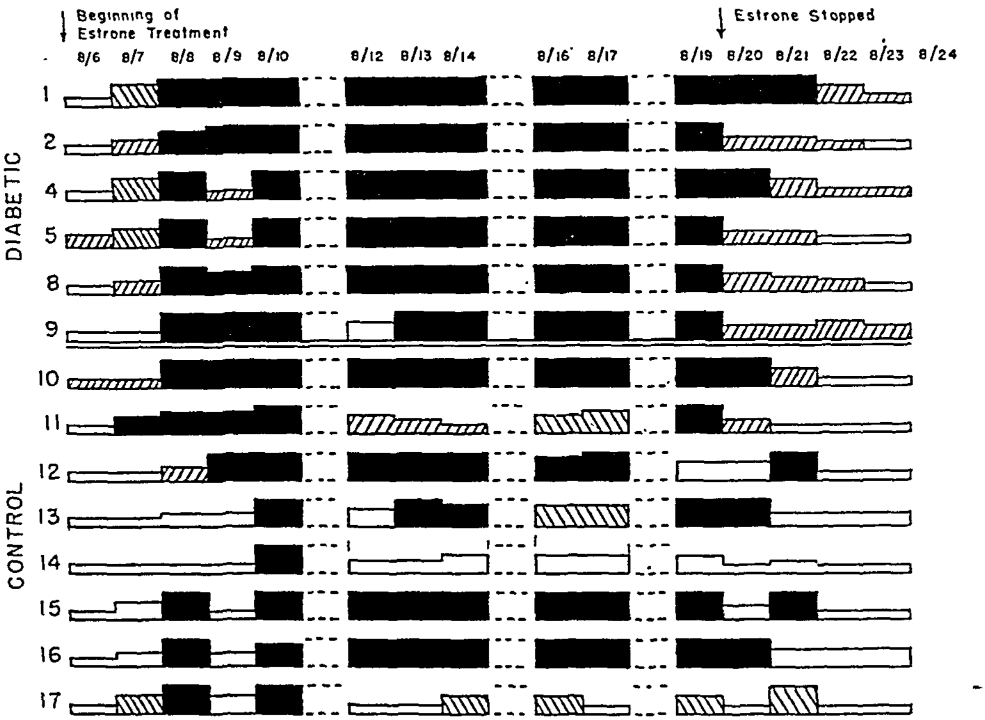

Fig. 1. The effects of estrone upon the vaginal cornification of diabetic and non-diabetic ovariectomized rats:
(solid blocks) cornified cells; (left diagonal) nucleated cells; (right diagonal) mixed cells; (blank) typical diestrous vaginal smear.

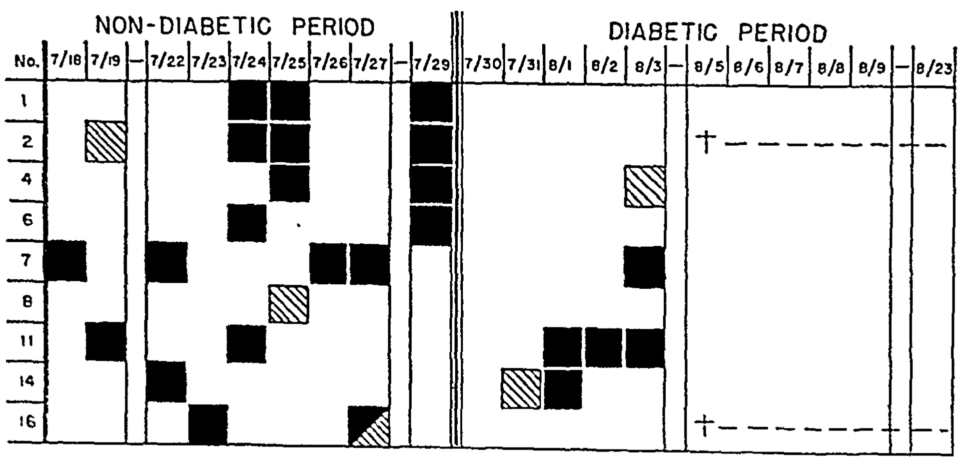

Fig. 2. The effects of diabetes upon the estrous cycles of adult rats:
(solid blocks) cornified cells; (left diagonal) nucleated cells; (cross) death of animal

trophin decreases were independent of other pituitary function. Two approaches to adrenal hypertrophy were used, the first by unilateral adrenalectomy and en-

suing hypertrophy of the remaining adrenal in diabetic and non-diabetic rats, and the second by comparison of regeneration of adrenals from the capsule after adrenal enucleation.

For the first experiment young adult female rats were used. The left adrenal glands were removed and weighed. The average adrenal weights for the two groups were 17.7 and 18.6 mgm. Then alloxan in 50–60 mgm./kgm. doses was given by intravenous administration to the first group. Blood glucose determinations were made every second or third day during the experimental period. The rats were autopsied on the 12th day following unilateral adrenalectomy and alloxan administration. The average weights of right adrenal glands in diabetic and non-diabetic groups were 32.2 and 33.8 mgm. respectively. It can be seen that the intact right adrenal gland of the diabetic rats hypertrophied to as great a degree in absolute units as did the intact adrenal gland of non-diabetic rats; the relative hypertrophy (expressed as milligrams per 100 grams of body weight)

TABLE 6

Hypertrophy of adrenals in diabetic and non-diabetic rats after unilateral adrenalectomy

NO. OF RATS	BODY WT. (GM.)		BLOOD GLUCOSE	ADRENAL WT. (MGM.)			PIT. WT.
	Beginning	Ending		Left excised	Right remaining	% difference	
			mgm./100 cc.				mgm.
6	137	126	381	17.7 (13.0)*	32.2 (25.5)	81.9 (96.1)	8.0
12	136	144	136	18.6 (13.7)	33.8 (23.0)	78.5 (67.9)	10.4

* Expressed as mgm./100 grams of body wt.

was greater in diabetic than in non-diabetic animals (table 6). The pituitary weights of the diabetic rats were smaller than those of the non-diabetic rats, as had been noted in the other experiments.

Adrenal enucleation was performed in young adult female rats, using the dorsal approach (table 7). An incision was made in the capsule of the adrenal gland and the cortex and medulla were removed by gentle pressure. At the time of adrenal enucleation some of the animals were given diabetogenic doses of alloxan (50–60 mgm./kgm.) by intravenous injections. All the rats were maintained on 1 per cent saline as drinking water for 10 days; thereafter tap water was used. Blood glucose determinations were made every second or third day throughout the experiment. The rats were autopsied 3 weeks after the adrenal enucleation and the regenerated adrenal cortex was dissected out and weighed. For two groups of diabetic rats the adrenal weights were 30.2 and 29.2 mgm. and for two control groups the corresponding adrenal weights were 29.5 and 30.2 mgm. The results of the experiment indicate that adrenal regeneration in the diabetic rats was equal to that in non-diabetic rats on an absolute weight basis, and slightly greater on a relative basis (mgm./100 grams of body wt.). Statistical analyses

indicated that no significant differences existed between diabetic and non-diabetic adrenal weights either after unilateral adrenalectomy or adrenal enucleation. Significant ($p = <0.05$) and highly significant differences ($p = <0.01$) were found between the diabetic and non-diabetic rat pituitary weights for the two experimental procedures.

The results of the experiments in unilaterally adrenalectomized and adrenal-enucleated diabetic and non-diabetic rats give evidence that the adrenotrophic function of the pituitary gland is unimpaired in diabetic rats, in contrast to the marked impairment of gonadotrophic function of diabetic rat pituitary glands.

Kraus (8) studied clinical material from diabetic patients and reported a clear decrease in the weight of the hypophysis, greatly diminished eosinophils both as to number and size, an increase of the "main" cells, and an apparent hydropic degeneration of the basophils. Serious changes in the ovaries of diabetic patients, consisting of "atrophy of the primordial follicles and corresponding lack

TABLE 7

Adrenal enucleation and regeneration in diabetic and non-diabetic rats during 3 weeks

NO. OF RATS	BODY WEIGHT (GMS.)		BLOOD GLUCOSE	ADRENAL WEIGHT	PITUITARY WT.
	Beginning	Ending			
			mgm./100 cc.	*mgm.*	*mgm.*
5	148	149	400	30.2 (20.3)*	9.1
5	145	165	148	29.5 (18.0)	13.6
12	132	145	323	29.2 (20.0)	8.9
14	135	159	120	30.2 (18.3)	12.0

* Adrenal weight in mgm./100 grams body weight.

of follicle maturation and absence of corpora lutea" were described. He further reported "a striking decrease in weight" in 2 of 3 pairs of ovaries of grown women. The changes in the ovaries studied by Kraus are entirely similar to those found in the ovaries of diabetic rats, and the diminished weights of both pituitary glands and ovaries are in accord with the findings presented here for experimental diabetes in rats.

Pituitary gonadotrophic dysfunction in diabetic rats has been noted previously by Shipley (5) in single diabetic rats, and in rats in parabiosis with non-diabetic partners. Both FSH and LH factors appeared to be affected. The data presented here extend and confirm the earlier observations concerning pituitary abnormalities in experimental diabetes (Shipley, 5). Earlier preliminary experiments indicated that insulin can reverse the gonadotrophic deficiency of the pituitary glands of alloxan-diabetic immature rats, and the work will be extended to cover experimental groups similar to those included in this paper.

SUMMARY. 1. Ovarian hypertrophy after unilateral ovariectomy was observed in young adult diabetic rats. The weight increases were 25 and 36 per cent for diabetic rats as compared to 147 and 91 per cent for non-diabetic rats.

2. Crude saline extracts of beef pituitary glands gave an ovarian weight increase of 52 per cent in non-diabetic and 48 per cent increase in diabetic rats over the corresponding untreated groups. The diabetic rat ovaries, untreated and treated with APE, were 20 per cent and 22.6 per cent less than those of corresponding non-diabetic groups. This difference was attributed to differences in endogenous gonadotrophins of diabetic and non-diabetic rats.

3. Crude APE given to rats for 5 days during starvation resulted in 17 and 84 per cent absolute increases over untreated fasted rat ovaries (30 and 49 per cent increases on a relative basis, i.e., mgm./100 grams of body weight).

4. Purified FSH preparations administered to diabetic and non-diabetic rats gave greater ovarian weights and greater percentage increases than did equivalent doses in non-diabetic animals.

5. Estrone administrations to ovariectomized immature diabetic rats gave equal or greater responses in diabetic than in non-diabetic animals.

6. Adrenal hypertrophy after unilateral adrenalectomy in diabetic rats was equal to the hypertrophy in non-diabetic, unilaterally adrenalectomized rats.

7. Adrenal enucleation in diabetic rats was followed by adrenal cortical regeneration to an extent equal to that in non-diabetic adrenal-enucleated rats.

8. Ovariectomized diabetic rats given 1γ estrone per day had more complete vaginal cornification than did non-diabetic ovariectomized rats given equivalent doses of estrone.

9. Rats that had regular estrous cycles became acyclic after alloxan-diabetes was induced; 4 of 9 rats had single estrous cycles within 5 days from the time of alloxan injection.

CONCLUSIONS

Untreated diabetic rats have greatly impaired pituitary gonadotrophic function. Rats recently made diabetic respond to exogenous gonadotrophin and estrogen. Pituitary adrenotrophic function appears to be unimpaired in diabetic rats.

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A QUANTITATIVE STUDY OF THE EFFECT OF HYPERTHYROIDISM ON GENITAL STRUCTURE AND FUNCTION

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Diverse views have been expressed concerning the functional relationship between the thyroid gland and the gonads. Some workers (Zawodowsky, 1927; Da Costa and Carlson, 1933; Smelser, 1937–1938) have reported that gametogenic activity is impaired in hyperthyroidism, while others (Hoskins, 1916; Iscovesco, 1913; Crew, 1925; Kunde, Carlson and Proud, 1929) have presented evidence suggestive of increased gametogenic activity. Several other workers (Schneider, 1939a, 1939b; Hamblen, Pullen and Cyler, 1941; Richter, 1944) would interpret this diversity as due to imbalance of katabolic and anabolic processes within the germinal tissue. In the present study our primary objective was to obtain quantitative and more reliable data on the effect of hyperthyroidism on genital structure and function than has been obtained in the past. At the same time, because of the nature of our experimental approach, it seemed desirable to include some observations on the effects of ligation per se on the genital tract structure and function.

Materials and methods. Sexually mature male albino rats weighing between 240 and 305 grams each were used. By random selection twenty-four rats were separated into four experimental groups having the same living conditions and same diet.

Seven animals comprising group I were subjected to bilateral ligation of the vas deferens, with the ligatures located in the proximal segments of the vasa near their junctions with the epididymi. Seven animals comprising group II were subjected to the same operation as the group I animals but in addition were administered 30 mgm. of desiccated thyroid (Armour) daily by stomach tube for ten successive days, beginning with the third post-operative day. Five animals comprising group III were subjected to mock-ligations and beginning with the third post-operative day were given 30 mgm. of desiccated thyroid daily for ten successive days by stomach tube. Five animals comprising group IV were subjected to mock-ligations only and retained as normal controls.

In all cases at the end of the thirteenth post-operative day, the testes and epididymi were removed and weighed separately. Immediately thereafter they were fixed for microscopical study. Individual body weight changes were recorded daily throughout the experimental period for use in indicating the relative degree of hyperthyrosis in those groups receiving thyroid. All data was tested by Fisher's formula (1928) for small samples and found reliable.

Results. The quantitative measurements obtained on body weight and on the gonads and epididymi of each experimental group are summarized in the accompanying table and are self-explanatory.

Microscopic findings. Peri-tubule edema of and disorganization of the ger-

minal epithelium of those parts of the seminiferous tubules adjacent to the rete testis characterized the testes of the ligated-normal animals (group I). Although from a histologic standpoint the degree of damage to testis structure and function could not be ascertained satisfactorily, the loss in testis weight shows that these organs suffered some pressure atrophy due to the ligation of the vasa deferentia (table). The testes of the hyperthyroid-ligated animals showed the same pressure involvement as that occurring in the ligated-normal animals. They differed from those of the normal-ligated control group in that the germinal epithelium was composed predominantly of spermatids, sperm and secondary spermatocytes and that the seminiferous tubules rather uniformly contained more sperm. The testes of the hyperthyroid animals (group III) showed no obvious change from normal. The testes of the normal control group animals (IV) showed no peculiarities.

TABLE 1

	TESTES *GRAMS %	EPIDID. GRAMS %	TESTES & EPIDID. GRAMS %	BODY WEIGHT CHANGE IN GRAMS
I Ligated-normal	0.9551	0.5744	1.5304	18 grams increase
II Hyperthyroid-ligated	1.2764	0.6609	1.9387	8 grams decrease
III Hyperthyroid	1.2432	0.4078	1.6512	1 gram increase
IV Normal	1.1462	0.3440	1.5053	16 grams increase

* Grams per 100 grams of body weight.

In all animals which were subjected to bilateral ligation of the vasa deferentia (groups I, II), spermatocysts were formed proximal to the sites of the ligatures and were filled with both normal and degenerating germinal products. All proximal levels of the epididymi contained abundant sperm with occasional immature forms. The epididymi of both the hyperthyroid (group III) animals and the normal controls (group IV) appeared completely normal.

Discussion. In the past, analyses of the rôle of the thyroid on gametogenic function have employed simple changes in weight of the gonads and the relative abundance of sperm in the testes and epididymi as criteria of the level of gametogenic activity (Hoskins, 1916; Zawodowsky, 1927; Smelser, 1934; 1937–1938; and Greenwood and Chu, 1939). These procedures do not take into consideration the fact that 1, the genital system is neither an anatomically nor a functionally closed system, and 2, the possibility of an alteration in the functional rate of the complex but highly effective mechanisms for transporting germinal products through the efferent genital ducts (Toothill and Young, 1931; Simeone and Young, 1931; Moore and Quick, 1924, et al.). It has been shown with regard to

the second point that sperm transference is notably increased in hyperthyroidism (Richter, 1944). It seemed to us that one must prevent this mass movement of germinal products through the efferent genital system if reliable significance regarding gametogenic activity of the testes is to be attached to changes in weight of the genital tract or to sperm abundance in hyperthyroidism. The only satisfactory method of accomplishing this is by ligation of the vasa deferentia. Comparison of the testis-epididymis weights of the normal-ligated animals with those of the hyperthyroid-ligated animals reveals that the latter increased 27 per cent above those of the normal-ligated control group animals. The major part of this 27 per cent genital weight increase was due to the effect of thyroid on the testes. This is especially significant because it means that those portions of the seminiferous tubules not suffering from pressure atrophy were markedly stimulated to over-activity by the induced hyperthyroid state.

The combined testis-epididymis weight of the hyperthyroid animals (group III) was 9 per cent above that of the normal control group (IV) but this difference statistically is just within the limits of reliability and can be disregarded. In either event the similarity in genital tract weights of these two groups (III, IV) is due to the relatively mild hyperthyrosis induced in the group III animals and also due to the fact that the germinal products are transported more quickly through the epididymis and vas deferens in hyperthyrosis (Richter, 1944). At the same time, it makes it doubly evident why sperm counts or simple testis or epididymal weights do not in themselves constitute accurate indices of gametogenic function in hyperthyrosis because they cannot and do not, in the absence of ligated vasa deferentia, provide a measure of those excess germinal products which, in a sense, overflow during the hyperthyroid state into the distal levels of the genital tract.

The effects as we observed them of vas deferens ligation (group I) on testis structure and function are in agreement with those reported by others (Nonidez, 1924–1925; White, 1932; Hotchkiss, 1944; Oslund, 1926; Moore, 1931) in that vas deferens ligation causes partial loss of gametogenic function due to pressure atrophy of portions of the seminiferous tubules and contributes to the formation of a spermatocyst at either the epididymal or vas deferens level. The literature bearing on the relation of the site of ligation to gametogenic impairment and spermatocyst formation is both controversial and vague (White, 1932; Van Wagenen, 1925; Moore, 1931). In view of the literature and of certain other unpublished data, it is suggested in order to avoid as much as possible impairment of gametogenic function and spermatocyst formation with its tendency to become fistulous, that in practice the site chosen for ligation be well distad in the vas deferens.

The findings that desiccated thyroid medication 1, raises the functional level of the mechanisms for transporting germinal products through the efferent genital tract, and 2, stimulates gametogenic activity in those portions of the seminiferous tubules not damaged by vas deferens ligation, are of clinical application in the reinstatement of normal genital tract function to previously vasectomized patients.

SUMMARY

1. The effects of hyperthyroidism on genital tract weight and structure of male albino rats with and without ligated vasa deferentia were studied.

2. Quantitative data and microscopic findings show that gametogenic function and the transference of germinal products through the efferent genital tract are increased in hyperthyroid states.

3. Ligation of the vas deferens near its junction with the epididymis induces partial degeneration of the seminiferous tubules but thyroid medication in such instances significantly increases the gametogenic function of the undamaged tubules.

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ACCLIMATIZATION TO EXTREME COLD

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The only unequivocal evidences of acclimatization to environmental conditions differing radically from normal temperate environments are those reported to occur in hot environments (4, 5, 12, 14). Data on the process of acclimatization to cold are lacking, although Bazett and his co-workers (1, 2, 16) have demonstrated slow adaptations to cool environments. A most remarkable example of adaptation to an ambient of approximately 0°C. has been shown to occur in the Australian aboriginal (6). Not only does he exhibit a greater lability toward vasoconstriction but also his heat production on exposure to cold remains at a constant level. On the other hand, according to Hill and Campbell (7), children receiving cold open-air treatment have elevated basal metabolic rates. Eskimos also exhibit BMR's from 14 to 21 per cent above normal standards (3). Animals have increased metabolic rates during and immediately after a period of continuous exposure to cold (11, 13, 15).

Most of these observations were made at relatively warm environments, freezing and above, and consequently do not provide the answer as to whether acclimatization occurs in the very extreme ambient temperatures observed in arctic and sub-arctic regions. Arctic explorers differ widely in their opinions both as to the occurrence and the rate of development of acclimatization.

In previous reports, Horvath and co-workers (9, 10) discussed the effects of short intermittent exposures to environments as low as −47°C. on the functioning of the heat regulating apparatus of men who either sat quietly or worked at a standard rate. Due to the nature of the observations, it was not possible to obtain evidence for or against the development of acclimatization to low temperatures. The data to be presented in this paper are concerned primarily with the reactions of men to an eight day period of continuous exposure to an ambient temperature of −29°C.

METHODS. A group of ten healthy young soldiers[4] were trained for twelve days outdoors in the July heat of Fort Knox, Kentucky. Their training consisted of walking a distance of 12 miles daily at a speed of 3.0 mph. Additional walks on the treadmill were also made daily. After this preliminary period, they were brought into the cold chamber described in another report (9) and remained there for three days in an envirionment of 25°C., R. H. 50 per cent. The chamber was then cooled to −29°C. Six of the ten men remained in

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[4] Average age, 21.5 years; height, 68 inches; weight, 155 pounds; and surface area, 1.83 square meters.

the cold room continuously day and night for eight days, while the remaining four slept in their barracks at night, but entered the cold chamber before breakfast each morning and remained there until after sundown each evening. They engaged in practically the same activities as the six men who resided continuously at −29°C. In the cold room the men wore the six piece Arctic Suit[5] M-1942, which has an insulative value of 3 to 4 Clo. Their daily activities for the three days previous to cooling the chamber, for the eight days at −29°C., and for the three days following the cessation of their low temperature exposure consisted of this set pattern: One hour's walk at 3.0 mph. after breakfast, then two hours of quiet sitting followed by lunch; after lunch, an hour's walk, a half hour's heavy work period, another hour's walk, a period of quiet sitting for 40 minutes, a series of psychological tests, and then dinner. There was some entertainment in the form of radio and reading during the day and motion pictures in the evening. Partial escape from the cold was permitted in the evening by the provision of a small hut in the cold chamber (temperature −5 to 0°C.). However, the men usually retired into their sleeping bags early in the evening.

TABLE 1

SUBJECT	AGE	HEIGHT	WEIGHT	SURFACE AREA (M^2)
		cm.	*kg.*	
FO	19	182	76.6	1.98
CU	20	175	70.6	1.85
RE	20	172	65.8	1.76
MO	19	165	56.6	1.64
BE	27	178	68.7	2.00

The results of studies on fluid balance, blood, and psychological responses (8) will be reported in later publications. The present paper is based primarily on observations of the sitting and working metabolism of five of the ten subjects. Only one of these five men was a member of the group which spent a portion of its time outside the cold room. The physical characteristics of these subjects are given in table 1. The procedures employed in this investigation were similar to those previously described (9, 10). The sitting metabolism of subject BE was obtained with a closed circuit apparatus and consequently for two hours he breathed air which was at a temperature of +20°C. For all the other sitting subjects the temperature of the inspired air was −29°C., the expired air being collected for ten minutes in Douglas bags. Analyses of aliquots of the collected air were made in duplicate on Haldane machines. Skin temperature data were incomplete, since the correct techniques for preventing breakage of the copper

[5] This consisted of two sets of ⅜ inch pile trousers, two sets of ⅜ inch pile parkas and a white cotton camouflaged outer suit. The inner pile suit was worn with pile towards the skin, while the pile of the outer suit faced the camouflaged garment. Two pairs of heavy wool socks were worn inside mukluks. Hand protection was provided by a pair of wool gloves and outer shell mittens (M-1943).

constantan thermocouples had not been fully developed. However, some data on toe temperatures were adequate and will be discussed. Rectal temperatures were obtained by means of calibrated clinical thermometers.

RESULTS AND DISCUSSION. The average rectal temperature of the five subjects throughout the day (measurements made on arising, after first work period, after rest period, after each of three afternoon work periods, and prior to retirement) was approximately 37.8°C. during the entire eight days at low environmental conditions. This was very similar to values obtained during the preliminary and the post-exposure periods. No significant changes were noted in the basal rectal temperatures secured while the subjects were still in their sleeping bags. Variations were noted in each subject but were not consistent. Basal values as low as 35.2°C. were found in a number of cases. The rate of fall of rectal temperatures during the sitting periods did not differ appreciably on any of the eight days. During the last third of the sitting period of the third day, there was observed a large fall in the rectal temperatures of all subjects, but its cause was not determined. Since adequate consecutive data on mean skin temperature were not obtained, changes in mean body temperature could not be estimated. In the few subjects on whom chest and thigh temperatures were successfully secured on all days at the low ambient temperatures, no changes attributable to duration of exposure were found.

The basal heart rate was not altered during the period of cold exposure. The mean values on the second day of cold were 56, on the fifth day, 55, and on the eighth day, 58. The heart rates during the sitting periods (table 2) were variable and might be explained as due to increased muscle tone or slight shivering by some of the subjects.

The mean oxygen consumption (open circuit method), during the two hours that four subjects sat quietly (fig. 1), showed an increase of approximately 30 per cent over control values at +25°C. Detailed data secured at three points during each two hour sitting period are given in table 1. No consistent pattern was exhibited during the eight days of exposure. While in general all subjects showed a trend to remain at an elevated level during the exposure to cold, two roughly similar groupings were noted in the daily variations of caloric expendi-iture. Nothing in the past experiences or the physical characteristics of the subjects could be correlated with these patterns.

After returning to a comfortable environment, the subjects' oxygen consumption decreased but was still higher than the pre-cold control values. This stimulating effect of cold exposure confirmed the results of animal experiments conducted by Horvath et al. (12).

It is of interest to compare the metabolic rates obtained on two subjects similarly exposed (fig. 2) when one of them, BE, breathed warm air while the other, FO, breathed cold air (−29°C.). Neither of these two subjects reported gross shivering, although they undoubtedly had some increased muscular tone. The increased metabolism was seen initially in both subjects, but after the first three days BE's heat production began to decrease, and in the last few days it was only slightly elevated. This response would be considered a classic example

of acclimatization to cold except that it was not noted for any of the four subjects who were breathing cold air. The after-stimulating effect of cold on metabolism was not observed to occur in subject BE. It is of special note that, while all the subjects reported they did not mind the cold so much after the first few days, only BE stated that he was definitely more comfortable. Whether he was the only subject to be acclimated or whether the breathing of warm air was the deciding factor was not then determined. Some experiments performed later

TABLE 2

The mean metabolic values obtained on four subjects who sat for a period of two hours each day during continuous exposure to the designated environmental temperatures

	ENVIRONMENTAL TEMP. °C										
	25.0°C.	−29.0°C.							25.0°C.		
		Days of exposure									
		1	2	3	4	5	6	8	9	10	11
At 40 minutes											
R.Q.	0.86	0.83	0.86	0.86	0.83	0.75	0.85	0.88	0.82	0.79	0.83
Ventilation L./min.	8.2	10.5	9.9	9.6	9.4	11.0	10.3	10.7	9.2	8.3	8.3
Oxy. cons. ml./min.	354	482	472	451	427	493	461	501	378	368	378
Cal./hr.	173	233	230	220	206	236	224	245	183	176	183
Rectal temp. °C.	37.2	37.0	37.3	37.4		36.7	37.0	36.5	36.6	36.7	36.9
Heart rate/min.	60	62	68	74	74	78	66	69	81	75	74
At 80 minutes											
R.Q.	0.89	0.88	0.82	1.04	0.86	0.90	0.86	0.85	0.94	0.92	0.80
Ventilation L./min.	7.5	8.5	8.0	8.4	9.0	12.0	9.4	9.8	9.5	9.9	8.2
Oxy. cons. ml./min.	307	358	368	316	381	503	374	416	303	309	324
Cal./hr	150	175	178	161	186	247	182	202	151	153	156
Rectal temp. °C.	36.7	37.1	37.2	37.1	36.9	37.1	36.9	36.6	36.9	36.8	37.0
Heart rate/min.	74	72	78	72	78	80	63	70	81	70	78
At 120 minutes											
R.Q.	0.88	0.82	0.81	0.88	0.85	0.86	0.83	0.92	0.82	0.80	0.82
Ventilation L./min.	7.4	9.4	9.2	9.5	9.7	10.2	9.4	9.6	8.5	7.9	7.8
Oxy. cons. ml./min.	300	400	426	419	442	455	424	420	354	330	332
Cal./hr.	136	193	204	210	215	222	205	208	171	158	160
Rectal temp. °C.	36.9	36.9	36.9	36.3	56.8	36.9	36.8	36.7	36.9	36.6	36.9
Heart rate/min.	66	64	61	65	59	75	72	73	74	76	70

failed to demonstrate any beneficial effects of breathing warm air during short periodic exposures to low environmental temperatures.

The toe temperatures obtained on BE and FO are of considerable interest (figs. 3a and 3b). They show the changes that occurred during the three hour morning periods (8 to 11 o'clock). Their toe temperatures were quite low from awakening through breakfast, but the walk raised them to fairly high levels. During the subsequent two hours of cooling, seated subject FO (fig. 3b) exhibited a strikingly similar pattern on all days, his final temperatures reaching the neighborhood of 10°C., similar to his pre-exercise levels. On the other hand, BE's toe temperatures presented definite evidence of an acclimatization effect

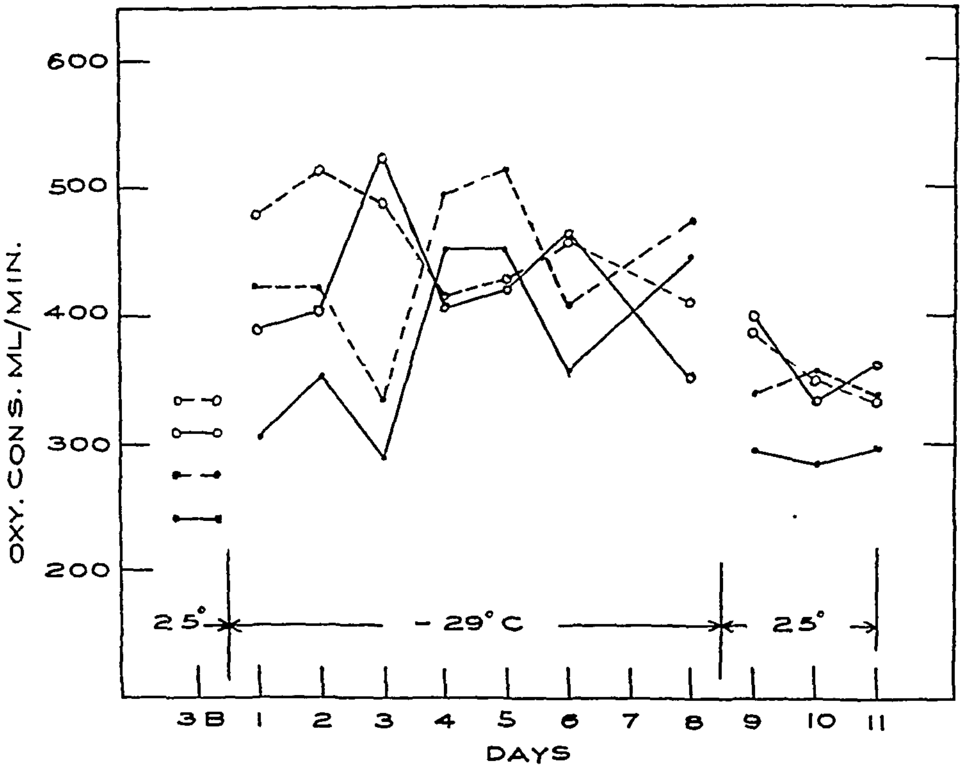

Fig. 1. The average oxygen consumption of four seated subjects during a two hour period in comfortable and cold environments.

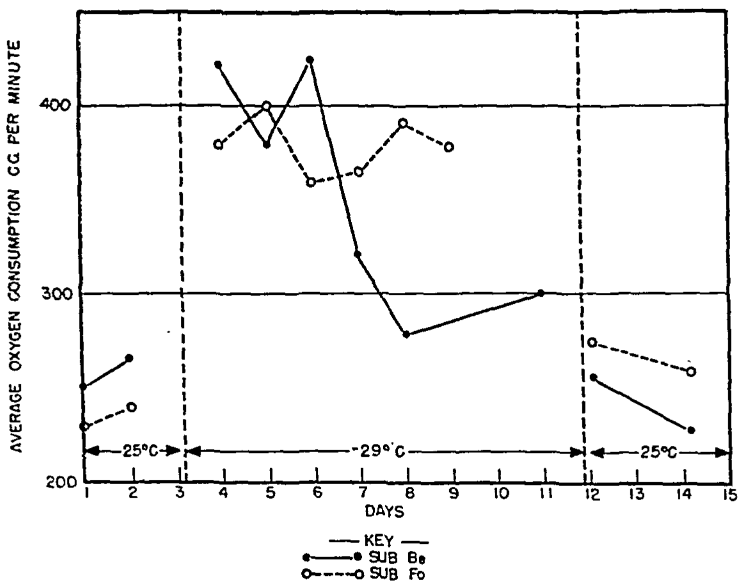

Fig. 2. Average oxygen consumption of two subjects sitting for two hour periods while residing continuously at the designated ambient temperatures.

in that the extremities not only warmed up more rapidly and to a greater degree on the last days of cold exposure, but also cooled at a definitely slower rate.

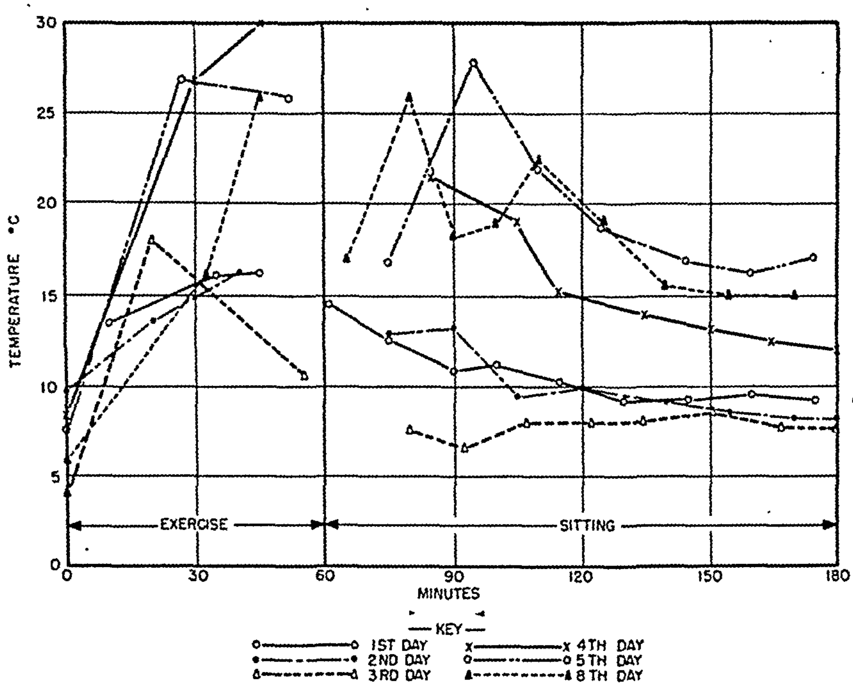

Fig. 3a. Toe temperatures of subject BE (one of the two subjects of fig. 2) during the morning hours of a period of continuous residence at a low environmental temperature.

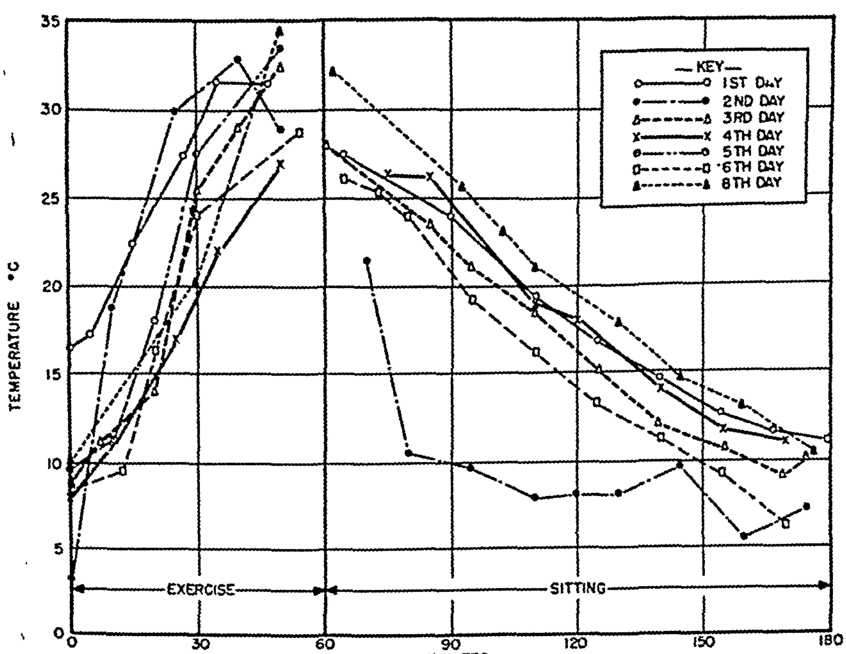

Fig. 3b. Toe temperatures of subject FO (one of the two subjects of fig. 2) during the morning hours of a period of continuous residence at a low environmental temperature.

There was a positive correlation between the higher final toe temperature and the mean caloric production during this cooling-off period. When his extremities

were cold, his metabolic rate was elevated and vice versa. If BE had been our only subject, definite evidence of acclimatization, in the usual interpretation of the word, again would be said to have occurred. However, his response was atypical in view of the reactions of the other subjects, and the breathing of warmer air may have altered his responses to some extent.

Metabolic observations were made on four subjects performing a standard amount of work at 25° and −29°C. Unfortunately, a follow-up period at 25°C. was not possible due to our having only one treadmill. Average data on four subjects are presented in table 3, with a graphic presentation in figure 4 of the findings on subject CU. As previously reported (10), exposure to low environmental temperatures was accompanied by an increased energy expenditure for the performance of a standard amount of work. The 25 per cent average rise

TABLE 3

Metabolic observations on four (4) men, dressed in Arctic clothing and walking on a treadmill at 3.0 MPH and a 3.3 per cent grade while exposed to an environmental temperature of −29.0°C. continuously for eight (8) days

ENVIRONMENTAL TEMPERATURE	VENTILATION		RESPIRATORY QUOTIENT		OXYGEN CONSUMPTION		HEAT PRODUCTION	
	L./min	Δ%		Δ%	L./min	Δ%	Cal/hr	Δ%
+25.0°C.	26.7		0.92		1.22		360	
Days at −29.0°C.								
First	33.2	24.3	0.86	−6.5	1.65	35.2	483	34.2
Second	31.1	16.5	0.87	−5.4	1.50	23.0	440	22.2
Third	30.9	15.7	0.88	−4.4	1.54	26.2	452	25.6
Fourth	32.4	21.3	0.90	−2.2	1.51	23.8	448	24.4
Fifth	32.6	22.1	0.86	−6.5	1.59	30.3	464	28.9
Sixth	31.3	17.2	0.89	−3.3	1.46	19.7	430	19.4
Eighth	29.8	11.6	0.86	−6.5	1.50	23.0	438	21.7
Average*....... ..	31.6	18.4	0.87	−5.0	1.54	25.9	451	25.2

* For days at −29.0°C.

in caloric output was slightly, but not significantly, higher in this group of subjects than in the group exposed for only a single hour to a similar ambient temperature (10). The energy expenditure during work decreased with continued exposure to the low ambient temperature. Although no regular pattern was evident in this increased efficiency of performance, viz., a return to cool environmental levels, the values obtained on the last two days were the lowest ones observed in the cold. Thus, continued exposure appeared to have a definite effect on physiological functions in that stimulation due to cold was less evident toward the end of exposure. This effect was not noted in all of the subjects (fig. 4). Except for his second day at −29°C., CU showed only minor changes in caloric output, which was partially a reflection of the slight rise in R.Q. observed in this subject.

The average oxygen consumption of the four subjects appeared to have reached

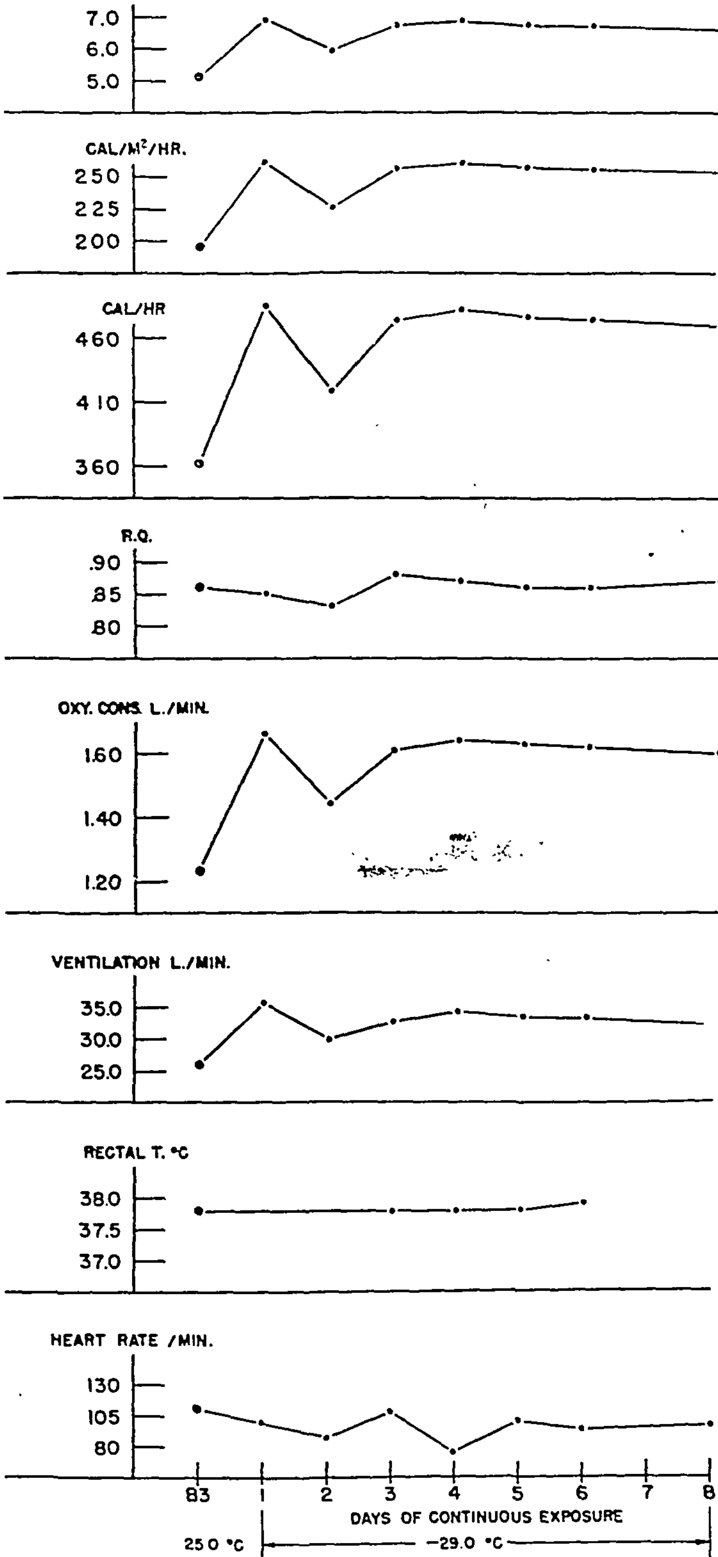

Fig. 4. Observations made on subject CU during a one hour walk (a.m.) on a treadmill at a speed of 3.0 mph and a 3.3 per cent grade before and during an eight day period of continuous residence in an environment of −29°C.

a fairly steady level after the first day in the low ambient temperature, the greatest variations being observed on the fifth and sixth days. The fall in R.Q. from the control days averaged about 5 per cent and showed minor variations during the succeeding days in the cold. The maximal fall occurred on the first day and, although it varied from day to day in the cold, it did not approach the pre-cold day values. Subject CU (fig. 4) again differed from the other subjects in that his R.Q. figures were generally higher during cold exposure than in the comfortable environment.

The ventilation rates at all times were greater at −29°C. than at 25°C. This increase, an average of 18.4 per cent, was accomplished through increased depth of breathing, as the respiratory rate was not affected. The smallest increase, approximately 12 per cent, was observed on the last day of the test. The greatest change was noted on the first day in the cold. Lower values, but not in a definite progression, were found on all succeeding days.

As illustrated in figure 4, the rectal temperature at the completion of the work period remained relatively constant during the successive days of cool and cold exposure. It is unfortunate that the constant breakage of thermocouples prevented collection of adequate data on the changes in body surface temperatures. The thigh temperatures obtained appeared to be higher on the last days than on the first days of cold exposure. The heart rate slowly decreased, the average fall being 12 beats. This may be in part a reflection of training.

Although the cost to the individual to do a given amount of work in the cold was always greater, a slight diminishing of this greater energy expenditure appeared to occur with longer periods of consecutive exposure to the low ambient temperature. This was reflected primarily in reduction of the total caloric output and the lowering of the minute ventilation volume.

Only one of the quietly sitting subjects exhibited measurable improvement in his ability to tolerate cold. All of the other men had variable responses and none of the changes in the physiological measurements could be attributed directly to length of exposure. It is possible that the duration of exposure was too short and that changes similar to those seen in subject BE may have occurred if the exposure to the cold had been continued.

There are indications that men do become acclimated to cold and that certain physiological mechanisms are involved in this process. Unfortunately, the individual variations are so great that no clearly delineated statement of the mechanism can be given at this time.

SUMMARY

Metabolic observations were made on five subjects who resided continuously for three days in a comfortable environment, 25°C., for eight days in a cold environment, −29°C., and for another three day period at 25°C. No changes in basal values for heart rate or rectal temperature occurred. The caloric expenditures during quiet sitting and while performing a standard amount of work were higher during exposure to the low ambient temperature. The duration of exposure to low temperatures did not markedly influence the energy output during the sitting period for four of the five subjects. The fifth individual, who

was breathing air of approximately 20°C. during this time, showed a striking decrease in caloric output with increased exposure. The significance of this finding and its association with a higher level of toe temperature has been discussed. Four of the subjects exhibited an increased metabolic rate—an afterstimulating effect of cold—on their return to the control environment. This was not observed in the fifth subject, the individual mentioned above.

The energy requirements for the standard work on a treadmill at 3.0 mph and a 3.3 per cent grade were increased during low temperature exposure. A small but definite return towards normal values occurred with continued exposure, but its relation to the development of a state of acclimatization was not clear. The decrease could be explained adequately on the participation of a number of other factors.

There is some indication from the data accumulated in this study that acclimatization to cold may occur, but at the present the evidence is too equivocal for a definite statement.

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THE FATIGUE OF STANDING[1,2]

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The greater fatiguing effect of continuous standing as compared with walking is a phenomenon of common experience (1) and observations from daily life indicate that work which is performed while standing produces fatigue more rapidly and to a greater degree than the same work accomplished while sitting (2). The mechanism which creates this difference in fatigue is obscure.

It is generally assumed that the usual balance oscillations of natural standing become increasingly unstable with fatigue although the opposite has also been reported. Military standing and so-called "A" posture reduce the sway as compared to that of the relaxed natural stance, and the oscillations may be further suppressed, or augmented, by the wearing of a pack (3). During pregnancy there is accruing postural instability (4). The effect of long hours of car and truck driving is to increase the oscillations of standing balance (5, 6). The steadiness of standing has been found to be closely related to the time of day (7, 8). Prolonged sleeplessness results in marked deterioration in the ability to stand erect with the eyes closed (7, 8). In a twenty-four hour investigation of the effects of cumulative fatigue upon standing, we have observed that the subjects first manifested an increase in balance oscillations which was followed by a marked decrease during the latter hours of the experiment. The aggregate of evidence suggests that the fatigue effect of standing may be dependent upon its quantitative factor.

The metabolic reaction to standing has been measured by numerous investigators. The cost of passive stance without muscular effort was found to be small and to vary insignificantly from the normal fluctuations of recumbency (9, 10, 11) as would be expected. The muscular contractions of active stance cause a rise in the metabolic rate (12). The cost may be increased still further by amplified balance oscillations; however, this is insignificant when compared with the high metabolic values for the muscular work of active movement (11, 13).

If the metabolic cost of standing is negligible, the accumulation of metabolites or the depletion of energy reserves, as the fatigue causative factor, apparently may be ruled out (11). If so, what then produces the sensation of exhaustion so characteristic of protracted stance? Continuous standing is exceedingly trying to some individuals, and persons who have difficulty in making and maintaining the circulatory adaptation for the change from recumbency to standing experience unpleasant sensations. Individuals have a characteristic reaction to quiet standing which is repeatable, and patterns differ markedly from one

[1] Aided by a grant from the Wisconsin Alumni Research Foundation.

[2] A preliminary report of this work appeared in Fed. Proc. March, 1947.

individual to another (14). The evidence of poor circulatory reaction is said to be reflected in a sensation of great fatigue or of dizziness (14, 15). In susceptible individuals the effective circulation may become sufficiently embarrassed to produce syncope (14, 15), and standing after exercise may be terminated by collapse due to orthostatic circulatory insufficiency (9, 16, 17). There are individuals whose circulation adapts with apparent ease to strenuous activity but who find quiet standing difficult (15) and there is no present means of predicting, or identifying fainters among medically and physically fit young men other than actual tiltboard testing (17). The physiologic reactions to the stress of vigorous activity and to the stress of continued standing appear to be separate phenomena, movement having better adaptation due to survival value than quiescence (15, 17).

It has been suggested that the sensation of stance fatigue is probably due to more or less acute hypoxemia of higher brain centers which exert a regulatory influence over muscle tonus and cardiovascular respiratory mechanisms (2, 11). If a subacute cerebral hypoxia produces the sensation of stance fatigue, might this be reflected in a measurable physiologic disruption of the stance equilibrating mechanisms which determine the magnitude and mean location of the balance oscillations during natural standing? To answer this question the present study was undertaken.

The purpose of this investigation was two-fold: to develop a method for producing fatigue of the antigravity muscles without untoward subsequent orthostatic circulatory insufficiency, and to demonstrate any effect of fatigue upon stance equilibrating mechanisms.

METHODS. The subjects of the study were 17 healthy young women, non-fainters with one exception, ranging in weight from 41.4 to 66.0 kgm., in height from 155.0 to 173.7 cm., and in foot length from 21.6 to 26.4 cm.

Standing and static effort were the methods of choice for inducing fatigue of the lower extremities. Static effort produces fatigue most readily and rapidly since the energy expenditure does not increase in proportion to the load and duration but more swiftly (18, 19). Preliminary experimentation showed that the duration and approximate load are measurable; that the effort may be maintained in the sitting posture with no untoward effects upon the circulation of non-fainters; and that it produces pulse rate and blood pressure responses comparable to those induced by moderate to hard muscular work.

The apparatus employed to determine experimentally the locations of the height of the center of gravity in recumbency, and of the center of gravity in the standing position has been presented in detail elsewhere (20, 21, 22).

The effect of standing and of static effort upon the height of the center of gravity. Standing. The subject stabilized comfortably relaxed in a chair near the balance table. Standardized recumbency was assumed upon the apparatus, the total recumbent height and the point of balance were determined. The subject then stood in a natural position for 15 minutes. The total recumbent height and the center of gravity were redetermined (table 1).

Static effort. After stabilization the total recumbent height and the point

of balance were determined. Assuming the sitting position, three five-minute bouts of static effort were performed with one minute of rest intervening. The subject then stood in a natural position for five minutes, then the total recumbent height and the balance point were redetermined (table 2).

TABLE 1

Effect of standing upon the height of the center of gravity

SUBJECT	RECUMBENT HEIGHT	HEIGHT OF THE CENTER OF GRAVITY					
		Before	After	Dif.	Before	After	Dif.
	cm.	*cm.*	*cm.*	*cm.*	*per cent*	*per cent*	*per cent*
RAA	166.2	91.9	91.6	0.30	55.29	55.11	0.18
	165.9	92.0	91.6	0.40	55.46	55.21	0.25
AMC	158.3	87.2	87.2	0.00	55.09	55.09	0.00
EC	173.7	96.4	96.2	0.20	55.50	55.38	0.12
CIE	166.2	92.5	92.1	0.40	55.65	55.41	0.25
	166.5	92.3	92.3	0.50	55.74	55.44	0.30
	166.2	92.8	92.7	0.10	55.85	55.77	0.08
	165.4	92.6	92.0	0.60	55.99	55.62	0.37
	165.7	92.4	92.0	0.40	55.76	55.52	0.24
MEE	170.3	92.7	92.2	0.50	54.43	54.12	0.30
ERH	167.2	93.0	92.8	0.20	55.60	55.50	0.10
MAH	159.3	88.0	87.6	0.40	55.24	54.99	0.25
PVK	169.6	94.7	94.4	0.30	55.84	55.66	0.18
EML	155.0	84.9	84.7	0.20	54.77	54.61	0.16
CMM	163.6	91.2	90.7	0.50	55.75	55.44	0.31
MS	173.0	93.7	93.0	0.70	54.22	53.75	0.47
HW	164.8	92.6	92.4	0.20	56.19	56.07	0.12
Averages				0.347	55.43	55.22	0.216

Results and their interpretation. Continued standing resulted in lowering of the balance point in 16 of the trials, with no effect on 1 individual. Amounts varied from 0.00 to 0.70 cm. Static effort induced transient lowering in 18 trials, with no effect in 3. The lowering ranged from 0.00 to 0.90 cm. The effect of static effort was slightly greater than that produced by continued standing; however, both resulted in transient lowering of the balance point in a significant proportion of the cases. This confirmed the results of earlier investigations (23, 24, 25). The shift in weight balance, although the individual

deviations were statistically insignificant, may represent considerable pooling of blood in the lower extremities.

The location of the height of the center of gravity in man has been of physiologic interest for over two centuries. Numerous investigators have studied the problem on the living, the cadaver, and the fetus. They originated and refined the method, devised different apparatus, and presented remarkably similar results. The height of the center of gravity from the soles of the feet was re-

TABLE 2

Effect of static effort upon the height of the center of gravity

SUBJECT	RECUMBENT HEIGHT	HEIGHT OF THE CENTER OF GRAVITY					
		Before	After	Dif.	Before	After	Dif.
	cm.	*cm.*	*cm.*	*cm.*	*per cent*	*per cent*	*per cent*
RAA	166.0	91.7	91.1	0.60	55.24	54.87	0.37
	166.0	91.2	91.2	0.00	54.93	54.93	0.00
EC	172.2	95.7	95.0	0.70	55.57	55.17	0.41
AMC	158.3	87.4	87.0	0.40	55.21	54.96	0.25
CIE	166.6	92.9	92.0	0.90	55.76	55.22	0.54
	166.2	92.6	92.3	0.30	55.71	55.53	0.18
	166.2	92.9	92.6	0.30	55.89	55.71	0.18
	166.2	92.3	92.1	0.20	55.53	55.41	0.12
	166.2	92.7	92.2	0.50	55.77	55.47	0.30
MEE	169.7	92.7	92.2	0.50	54.63	54.33	0.30
ERH	167.2	92.7	92.7	0.00	55.44	55.44	0.00
	166.9	92.8	92.4	0.40	55.60	55.33	0.27
	166.9	92.6	92.5	0.10	55.48	55.45	0.03
	166.9	92.7	92.3	0.40	55.54	55.36	0.18
MAH	159.4	88.0	87.6	0.40	55.21	54.96	0.25
PVK	169.6	94.9	94.0	0.90	55.95	55.42	0.53
EML	155.0	85.2	85.2	0.00	54.96	54.96	0.00
CMM	163.7	91.6	91.2	0.40	55.95	55.71	0.24
	163.7	91.4	90.9	0.50	55.83	55.52	0.31
JN*	160.2	87.3	86.8	0.50	54.49	54.11	0.38
MS	173.0	94.2	93.3	0.85	54.41	53.93	0.48
Averages...............................				0.421	55.39	55.13	0.253

* Subject with 6th lumbar vertebra

ported to be from 54.8 to 59.8 per cent of the total recumbent height. It is lowest in the male cadaver (26), higher in men (24, 27, 28) than in women (20, 28, 29), and highest in children (30) and in the fetus (30, 31). Respiration and heart-beat cause fluctuations in height, and standing, walking, and running result in graded transient lowering of the center of weight (23, 25, 32, 33). The lowering was attributed to a "blood shift" toward the active muscles reported in gram centimeters per kilogram of weight as 136, 255, and 471, the mean values for 56 subjects determined on a recording balance table (25). The possible "blood shift" to the vascular reservoir of the lower extremities was estimated to average at least 870 grams, or nearly 2 pounds of blood more than the normal amount (34). This has also been reported as a displacement of an average of 256 cc. of fluid during 15 minutes of quiet standing (9). The lowering produced in the height of the center of weight during standing, walking, and running reported in millimeters was 0.72, 2.21, and 5.38 (23). Our value for standing, 3.47 mm. (table 1) is higher but compares favorably and the data also contribute the additional information that the effect of static effort, 4.21 mm. (table 2), is comparable to that of active exercise. Although the deviations were small, they represent a considerable pooling of blood in the lower extremities which probably results in decreased blood flow to the head with subsequent hypoxia in higher neural centers. Changes in the height of the center of weight may also stimulate equilibratory mechanisms. It is therefore suggested that the weight redistribution, the accompanying stimulation of equilibratory mechanisms, and the hypoxia of neural centers may be component primary factors in producing the complex sensation of the fatigue of standing.

The effect of static effort upon the mean locus of the center of gravity oscillations during standing. The subject was seated comfortably relaxed on a stool beside the scale platform and allowed to stabilize. The subject then dipped the soles of the feet in potassium permanganate solution and stood naturally in a standardized foot position upon absorbent paper on the platform. A five-minute record of stance oscillations in the antero-posterior and lateral planes were made on a moving kymograph. Seated, with relaxed upper body and fully extended lower extremities, the subject supported a 4.5 kgm. sandbag placed across both ankles. Adhesive tape over the belly of the quadriceps femoris muscle held a silk thread taut to a straw lever which recorded any muscle tremor on a moving kymograph during the muscular effort. The static contraction was maintained for five-minute periods with one minute of rest intervening until unbearable pain terminated the bouts. The subject was quickly assisted to stance on the platform and five minutes of oscillation observations were made. Blood pressure and heart rate determinations were secured at 60 second intervals throughout the entire experimental period. The subject was blindfolded while seated to eliminate extraneous stimuli attendant upon operative procedures.

Results and their interpretation. The experimental data include 34 sets of 25 serial, center of gravity oscillation observations, 34 footprints, pulse rate and blood pressure determinations, and tremor kymograms.

Effects of static effort. Pulse rate and blood pressure observations served as

indices of the relative severity of the physiologic effect produced by static effort. The lowest resting level for pulse was 58 beats per minute, and the highest value during static effort was 120. The pulse rates increased from 12 to 56 beats per minute. An increase of 26 beats is reported to indicate moderate work, 33.5 beats hard work, and 54 beats exhaustive work (35). On the basis of this criterion the static effort may be classified from moderate to exhaustive, depending upon the reaction of the individual subject. The systolic blood pressure increased from 26 to 70 mm. Hg with static effort, and the highest reading obtained was 170 mm. Hg. These findings compare favorably with those of other investigators (12, 19).

The static effort was hard and difficult to maintain. The thigh muscles soon ached, frequently cramped, and the pain became severe with repeated bouts. The subjects averaged three bouts of effort. Relief from pain was instantaneous with relaxation. Paresthesia of the feet was subjectively reported. Perspiration often appeared on axillae, forehead, abdomen, and palms of the hands in spite of the two fans employed. The subject sometimes manifested irritability. Sighing and Cheyne-Stokes respiration were observed in some subjects. Fasciculating tremor of the quadriceps femoris was coincident with the static effort, increasing in amplitude with each bout, often appearing in related muscle groups and in other musculature. These findings agree with the report which also points out that fatigue of static effort was definitely associated with pain and muscle tremor, and that the recruitment of other sets of muscles was gradually included until the total musculature of the body seemed to be involved (19).

After the static effort every subject was able to tolerate five minutes of standing. One subject was markedly unstable almost to the point of falling, then suddenly steadied and maintained a stance resembling bipedal reflex rigidity (figs. 1 and 2). Since this subject habitually swayed considerably, the suppression of oscillation was a pronounced phenomenon.

The locations of the center of gravity oscillations during standing before and after static effort were calculated from the kymograms and plotted on the corresponding footprint records.

In the majority of individuals the mean location of the center of gravity oscillations in natural standing is normally eccentric back and to the left of the geometric center of the total base of support (36, 37). The eccentricity is a compensatory phenomenon which counterbalances the forwardly imbalanced and asymmetric weight distribution of the body (38). All of the subjects of this study were normally eccentric in the backward direction, with 67 per cent to the left of the geometric center and 33 per cent to the right. After static effort the usual mean location of the balance oscillations was consistently disrupted, the amount and direction being variable (table 3). It is known that stance oscillation patterns are highly individual and repeatable (39, fig. 2). Since this is true, and recognizing the individual variance in direction of weight displacement which occurred following static effort fatigue, the mass handling of these data could mask pertinent information concerning the physiologic reaction to equilibratory stress.

Analysis of the data. Since the successive determinations of the loci of a particular subject's center of gravity projection during pre- or post-static effort periods are serially correlated, these data cannot be analysed validly by customary statistical procedure, which assume independence of the successive determinations of the values of the parameters under investigation (40). The oscillations of the center of gravity baffle quantification due to the harmonic motion involved. These factors preclude the statistical analysis of the data

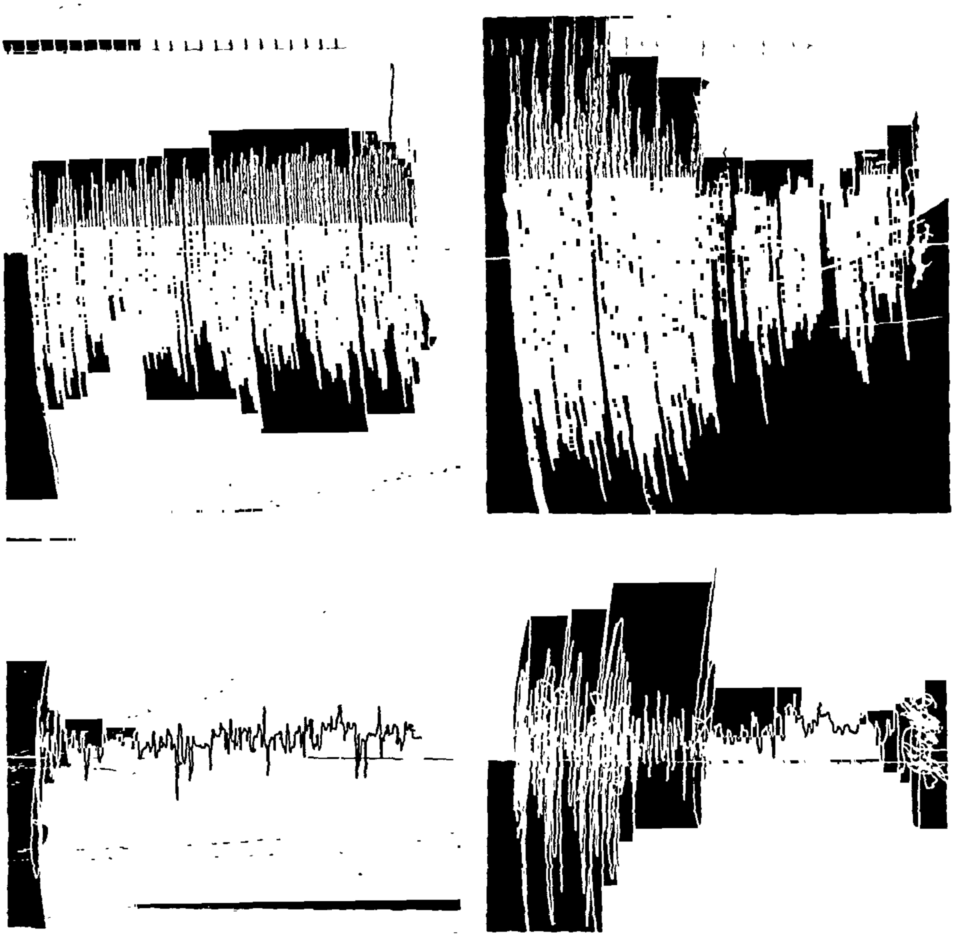

Fig. 1. Kymograms of synchronous bi-plane center of gravity oscillations during five minutes of natural standing before and after static effort.
Upper: anteroposterior; *lower:* lateral;

However, the bi-plane center of gravity oscillation kymograms provided the data for plotting the 25 serial observations on the corresponding footprint of each subject. The geometric center of the footprint then served as a benchmark for the orientation of the mean location of the oscillations.

Every subject manifested a change in the mean location of the oscillations after static effort during one, or more, sets of observations. Changes in both antero-posterior and lateral planes were exhibited by 55.6 per cent of the sub-

jects, 33.3 per cent in the lateral plane alone, and 11.1 per cent in the antero-posterior plane only. The disruption in the lateral plane appears to occur more readily than in the antero-posterior. Since the usual stance eccentricity is due in part to asymmetric weight distribution this would be expected. The lateral displacement was emphasized by one susceptible individual, affected only in this plane, who manifested the most marked imbalance of any subject, maintaining equilibrium with great difficulty immediately after static effort, as illustrated in figures 1 and 2. The evidence indicates that the mechanism

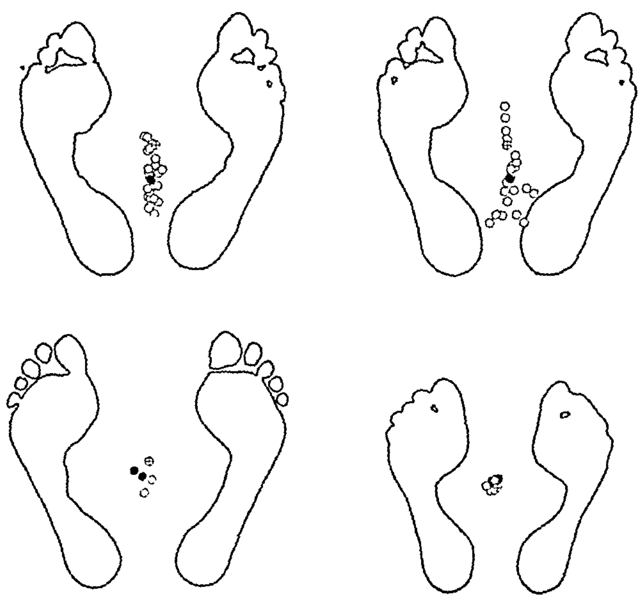

Fig. 2. Reproduction of footprints with projected center of gravity locations.

Upper left and right: before and after static effort center of gravity locations during five minutes of natural standing.

Lower left and right: the mean location of center of gravity oscillations during five minutes of natural standing before and after static effort projected onto the same footprint. *Left:* two sets of observations on MS. *Right:* four sets of observations on EML.

Crossed circle: geometric center of the base of support.

Open circle: upper footprints—instantaneous center of gravity locations.
lower footprints—mean of pre-effort oscillations.

Closed circle: upper footprints—mean of instantaneous locations.
lower footprints—mean of post-effort oscillations.

maintaining the usual compensatory eccentricity of stance is altered by fatigue of static effort. Two subjects were only slightly affected in one, or more, sets of observations. This may be accounted for by a high degree of physiologic efficiency in reaction to fatigue stress, by the effort being too easy for the subject, by individual differences in the ability to endure sensations of stress and in the willingness to push oneself beyond the threshold of discomfort.

In the static effort involving the antigravity musculature, as in continuous standing, there is probably an excessive generation of motor and sensory nerve impulses. The static contraction not only results from repetitious motor stim-

ulation, but also gives rise to sensory stimuli which produce the sensation of discomfort, these sensory stimuli elicit reflex stimulation of antigravity musculature and recruit contraction in other muscles (12, 19). Additional sensory

TABLE 3

The average deviation of the center of gravity from the geometric center of the footprint expressed in per cent of one-half the diameter in each plane

	ANTEROPOSTERIOR DEVIATION				LATERAL DEVIATION			
Subject	Pre-.	Post.	Dif.	A-P	Pre-.	Post.	Dif.	R-L
	per cent	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*	
EB	−3.85	+0.46	4.31	↑	−6.79	−6.65	0.14	→
MB	−4.67	−6.76	2.09	↓	−2.02	−3.35	1.33	←
RMC	−26.17	−29.27	3.00	↓	−8.94	−13.96	5.02	←
FAH	−25.80	−16.77	9.03	↑	−0.37	−0.31	0.06	→
	−23.90	−23.95	0.05	↓	−2.74	+3.84	6.58	→
PVK	−19.23	−16.48	2.75	↑	−2.32	−1.96	0.36	→
	−16.07	−9.29	6.78	↑	+3.27	+3.33	0.06	→
	−14.03	−17.59	3.56	↓	−0.59	+0.19	0.40	→
	−7.19	−23.29	16.10	↓	−0.18	−1.62	1.44	←
EML	−2.73	+9.09	11.82	↑	−2.91	−2.12	0.79	→
	+3.97	+9.00	5.03	↑	−4.70	−0.94	3.76	→
	−3.62	+1.64	5.26	↑	−7.07	−0.55	6.52	→
	+1.61	+5.11	3.50	↑	−10.76	−4.22	6.54	→
LML	−6.47	−18.75	12.28	↓	−2.21	+6.32	8.53	→
HMS	−10.63	−18.29	7.66	↓	−2.28	−5.41	3.13	←
MS	−15.12	−10.63	4.49	↑	+3.11	−4.61	7.72	←
	−24.35	−5.82	18.53	↑	−3.06	−10.77	7.71	←

+: in front, or to the right, of the geometric center
−: back, or to the left, of the geometric center
25 observations in each plane pre- and post-static effort
Average difference: Anterior 7.15
Posterior 6.39
Right 2.98
Left 4.39
Post-effort shift: Anterior-right 8 experiments
Anterior-left 2
Posterior-right 3
Posterior-left 4

stimuli are then produced which contribute to the sensation of discomfort which may vary from mild uncomfortableness to severe pain. Since relief from the pain of static effort is instantaneous with relaxation, this would suggest either

a circulatory or a nervous component, or a combination of both, with an excessive generation of sensory stimuli as the dominating factor.

Paradoxically, both extremely relaxed stance, and certain rigid postures, create no sensations of fatigue. Relaxed comfortable standing is reported to be nearly indefatigable, being accompanied by only slight sensations of discomfort (11). Decerebrate animals can maintain rigid postures for long periods of time without apparent fatigue, and catatonic patients can hold unnatural static positions for longer duration than normal individuals can tolerate without fatigue (12). The lack of apparent fatigue may be accounted for in the decerebrate animal if sensory stimuli are unable to bombard higher nerve centers which register pain sensations, and in the catatonic patient by the inability to experience normal sensations of pain, or fatigue. It is possible, therefore, that the apparent indefatigability of rigid posture may be due to lack of perception of the excessive generation of sensory stimuli, and that the economy of muscular contraction during exceedingly relaxed comfortable standing may not produce excessive volleys of sensory stimuli which reach consciousness as sensations of fatigue.

Stance fatigue: a release phenomenon. The disadvantages of standing were recognized by Holland who is quoted by Runting (41) as pointing out that "standing combined all the fatigue of walking with the *stagnation*[3] of rest". Due to the effects of gravity a greater amount of blood descends into the lower extremities of the body in standing, than in sitting; because of this, the upper circulatory half is deprived of a not inconsiderable quantity of blood, of which the supply to the brain is first to be diminished (42). It is common knowledge that functioning parts of the body are more vascularized during activity than at rest. The sustained muscular contraction of static effort probably increases the vascularization to the muscles involved. The circulating volume of other parts is thus decreased. With the subsequent assumption of the erect posture, gravity causes a further increase in the blood volume of the lower extremities, resulting in a greater depletion of circulation through the head. The brain is particularly sensitive to oxygen lack. It is probable that the decreased supply of blood to the brain becomes noticeable as a slowly increasing aggravation and disturbance of the innervation and association processes decurrent in the brain (2). The motor impulses can no longer be sent out to working musculature in the same precise and finely graduated manner. The antigravity, and other, muscles become more innervated than is necessary for the effort, and the stance oscillations become altered. Every muscular effort presupposes an established innervation and control function of the brain, therefore, it is quite possible to trace the early onset of fatigue at work while standing to cerebral hypoxia evoked through standing (2).

Presupposing that when the narrow tolerance to oxygen depletion is reached in higher brain centers governing equilibrium, the inhibitory control over lower, less vulnerable, mechanisms is lifted, and an induced state similar to decerebrate rigidity, or bipedal reflex standing, is thus initiated. In the transition stage the fluctuations of neural control may produce instability of equilibrium

[3] Italics by author.

which may, or may not, eventually resolve into variable degrees of temporary rigidity of stance. When this occurs, the stance oscillation pattern changes. The evidence presented in this study indicates that some dominant change in the equilibratory mechanism markedly disrupts from its usual position the mean location of the center of gravity oscillations during natural standing when fatigued. Perhaps the normal compensatory eccentricity of weight is affected, or lost, through lessened higher control superimposed upon more primitive stance mechanisms during the process of evolution, and older patterns of equilibrium tend to reappear. It is known that disease releases equilibratory mechanisms from higher neural control, therefore it seems reasonable to assume that stance fatigue due to subacute cerebral hypoxia might affect vulnerable higher equilibratory mechanisms and induce a similar release phenomenon.

Stance fatigue: an excessive accumulation of sensory stimuli. Grow (43) believes that "fatigue is due to an excessive generation of nerve impulses and originates in the nervous tissues of the body". That "it is the act of generating, of creating, of producing the nerve impulse that is exhausting—and not the muscular exertion that causes fatigue". The hypothesis is therefore suggested: that protracted standing evokes an excessive generation of nerve impulses which cumulates in the sensation of stance fatigue. This is initiated through repetitious stimulation of antigravity musculature and through subacute cerebral hypoxia which lifts higher neural inhibitory control over lower equilibratory mechanisms, thus releasing excessive stimuli to antigravity muscles. The tonic antigravity musculature, in turn, gives rise to volleys of sensory stimuli which produce sensations of discomfort, and which find neural expression in efferent stimulation to other musculature, with subsequent contraction and sensory reaction. Thus an additive sensory resultant is created which impinges on consciousness as the fatigue of standing.

SUMMARY AND CONCLUSIONS

The purpose of this study was to investigate the fatigue of standing by developing a method for fatiguing the lower extremities and by demonstrating any fatigue effect upon physiologic mechanisms regulating the height of the center of gravity and the mean location and magnitude of the balance oscillations during natural standing.

Fatigue was produced by continued standing and also by static effort. The effort consisted of full extension of the lower extremities while supporting a 4.5 kilogram sandbag during three or more five-minute periods with one minute of rest intervening.

Seventeen determinations of the effects of continued standing upon the height of the center of gravity were made on 12 young women, and 21 observations of the effects of static effort upon the height of the center of gravity were made on 17 young women.

The effects of static effort upon the mean location and magnitude of the center of gravity oscillations during natural standing were observed on 9 young women, yielding 1620 single center of weight determinations.

The following conclusions have been reached:

1. The height of the center of gravity of the body is lowered by continued standing, and by static effort involving the lower extremities.

2. The usual mean location of the balance oscillations of the center of gravity of the body during natural standing is displaced by the fatigue of static effort performed by the lower extremities.

3. The fatigue of static effort produces an increase in the magnitude of balance oscillations, a decrease in magnitude, or a combination of both.

4. The lowering of the center of weight and the marked change in the magnitude and mean location of balance oscillations suggests a weight redistribution of circulatory origin.

5. Subacute cerebral hypoxia may release lower equilibratory mechanisms from higher inhibitory control, thus producing hypertonia of antigravity musculature.

6. It is suggested that the redistribution of fluid weight, the protracted generation of motor stimuli producing atonicity of antigravity and other musculature, and the excessive volleys of sensory stimuli thus elicited, probably create an additive resultant expressed as the fatigue of standing.

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RELATIONS BETWEEN CUTANEOUS BLOOD FLOW AND BLOOD CONTENT IN THE FINGER PAD, FOREARM, AND FOREHEAD[1,2]

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Decreases in limb or organ volume have frequently been interpreted as indicative of increased arterial tone or decreased blood flow. The possibility of the changes in volume of an extremity being due to reactions on the venous side of the vascular bed has been indicated in studies where the necessary correlations between volume and flow could be made (1, 2). A similar examination of these relations in any skin region may be effected by photoelectric plethysmography (3). The necessary data are available in the photoelectric plethysmogram since the simultaneous changes in blood content and blood flow can be estimated through the calibration of the photoelectric plethysmogram as described below.

This paper describes, first, the application of the photoelectric plethysmograph to the estimation of changes in blood content and of the level of the blood flow in the skin, second, the correlations between flow and volume changes; third, the use of these correlations in estimating the directional changes in arterial and venous tone, fourth, the estimation of the blood depot in the minute vessels of the skin.

METHODS. 1. *Changes in the blood content of the skin* were estimated with the photoelectric plethysmograph by calibrating the photoelectric plethysmogram in terms of the "filter unit" and then estimating the volume equivalent of the latter.

a. *Calibration of the photoelectric plethysmogram in terms of the "filter unit".* Previous descriptions of the technique of photoelectric plethysmography (3, 4, 5) have referred to a method of calibrating the plethysmogram in terms of an arbitrary but reproducible unit called the "filter unit" (fig. 5, reference 4). This is simply the deflection in the photoelectric record produced by insertion of a clear glass plate (the filter) in the path of the light returning to the phototube. We may then express the deflections in the photoelectric record due to changes in blood content in terms of "filter units" by using the equation

$$\Delta V_F = \frac{\Delta V_l}{F_i} \tag{1}$$

where F_i is the deflection in the photoelectric record due to the insertion of the filter; $\Delta\ V_l$ is the deflection due to a change in blood content (increase or de-

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[2] Aided by a grant from the American Medical Association.

[3] Now at the University of Kansas, Lawrence, Kansas.

crease); and ΔV_F is ΔV_I expressed in filter units. Obviously, this procedure is applicable to the measurement of either the amplitude of the volume pulse or other changes in the blood content of the skin. Our problem, then, in quantitating the photoelectric plethysmogram, is to determine the volume equivalent of the "filter unit".

b. *The estimation of the volume equivalent of the "filter unit".* Comparisons of mechanical and photoelectric plethysmograms which were recorded simultaneously from the same finger show qualitative parallelism in the two records. By appropriate adjustments in recording sensitivity, one may superimpose one record upon the other. This fact suggests that the mechanical plethysmogram may be employed for the calibration of the volume changes as inscribed by the photoelectric plethysmograph. Difficulties arise, however, for the reason that the mechanical plethysmogram also includes changes in volume which may involve the larger vessels of the finger; similar effects are absent from the photoelectric plethysmogram since it reflects only those changes in volume which are occurring in the minute vessels of the skin.

The fact that the volume changes in the photoelectrically recorded plethysmogram of the finger pad are *relatively* smaller than those in the mechanically recorded plethysmogram of the finger is readily demonstrated by calculating the ratio of the reduction in volume to the decrease in blood flow during a reflex vasoconstriction. Since the amplitude of the volume pulse varies directly with the blood flow (5), it is convenient for the purpose of calculating the value of this ratio in both plethysmograms to substitute the amplitude of the volume pulse for blood flow. Further, although the two plethysmograms are expressed in different units (the photoelectric in terms of "filter units" and the mechanical in terms of volume), it is still permissible to compare the values of the ratios from the two records since the "filter unit" may be defined in terms of its volume equivalent. The ratio of change in volume to change in flow is about twice as large for the mechanical plethysmogram when this is recorded from the terminal phalanx of the finger as for the photoelectric plethysmogram recorded from the skin of the finger pad. Inclusion of still larger vessels by recording the mechanical plethysmogram from the entire finger further increases the difference between the two ratios.

The error introduced into the comparison of the photoelectric and mechanical plethysmograms in the manner indicated above would be eliminated in a comparison of the cutaneous volume pulses as recorded by the two methods, since the volume pulses are exhibited only on the arterial side of the circulation under normal conditions. The volume pulses of the terminal phalanx were recorded by enclosing it in a light metal capsule (a heavy automobile grease was used to effect a seal) and by using air transmission to an optical recorder. Tests of the frequency of response of the system indicated that it was adequate for the purpose of calibration. Both the volume and the surface area of the phalanx within the capsule were carefully measured, the former by the displacement method, the latter by taping and then measuring the area of the tape. The simultaneous photoelectric values of the mean phalanx skin pulses were esti-

mated from the photoelectric records according to the procedure which is described in equations 2 and 3 and table 5 of reference 5. The equivalence of the mechanical and photoelectric values is expressed in the equation

$$(2)\ V.P. = K_v \cdot P_{PH}$$

where $V.P.$ is the amplitude of the mechanically recorded phalanx volume pulse in cc./cm.^{2} skin; P_{PH} is the photoelectrically estimated amplitude of the phalanx volume pulse expressed in "filter units" (see equation 3 of reference 5 for the method of calculating P_{PH}); and K_v is the volume equivalent of the "filter unit" in cc./cm^2. The determination of the value of K_v provides a method for applying a calibration scale to the photoelectric plethysmograms recorded from various skin regions. Table 1 summarizes such a determination of K_v on 4 subjects. The average value of K_v, thus determined, is 0.00026 cc./cm.^{2} skin/filter unit.

TABLE 1

The volume equivalent of the "filter unit"

SUBJECT	V. P. VOLUME PULSE OF TERMINAL PHALANX	P_{PH}*	$\frac{V.P.}{P_{PH}}$ K_v cc./cm.^{2}/filter unit	NUMBER OF OBSERVATIONS
	cc./cm.^{2} skin	*"filter units"*		
La	0.000135	0.51	0.000264	6
Mi	0.000243	0.98	0.000249	4
Ki	0.000180	0.68	0.000265	10
	0.000500	1.98	0.000252	6
T.J.	0.000175	0.67	0.000261	15
Average			0.000260	

* P_{PH}—average phalanx pulse as estimated photo-electrically (see equation 2, reference 5).

Subjects are the same as in table 5, reference 5.

The calibration of the photoelectric plethysmograph in terms of changes in blood content is then obtained from the relation

$$(3)\ \Delta V = 0.00026 \cdot \Delta V_P$$

where ΔV is the change in blood content of the skin (cc./cm.^{2} of skin) and ΔV_P is the deflection (expressed in "filter units") in the photoelectric record due to the change in the blood content of the skin. This relation applies to either an increase or a decrease in blood content of the skin.

One cannot be certain of the absolute accuracy of this experimentally determined value of K_v since exact equivalence of the volume pulse of the phalanx and the photoelectrically estimated skin pulse of the phalanx may be affected by the inclusion of the pulse of the digital artery in the mechanical plethysmogram. This error cannot be large, however, for the following reasons: first, the per cent change in the *volume pulse* of the phalanx during vasomotor reflexes is approxi-

mately the same as that in the photoelectrically recorded skin pulse; second, the digital artery does not participate in these reflexes (6); it cannot then contribute significantly to the phalanx pulse.

The choice made in this paper of expressing the changes in blood content of the skin in terms of surface area is guided by several considerations. It is most convenient to express the cutaneous blood flows in terms of surface area and it is also necessary to do so in regions other than the digits since only in the latter may one estimate skin volume. The quantitative study of the relations between blood flow and blood content (see below) necessitates similar units in measurement. The actual volume of skin which is to be utilized in the ratio of vascular volume to skin volume is indeterminate. The surface area is readily measured.

2. *The blood flow in any skin region* may be estimated from the equation

$$(4)\ \text{Flow/minute} = K_f \cdot P_{PH}$$

where

P_{PH} is the amplitude of the photoelectrically recorded cutaneous volume pulse expressed in "filter units";

K_f is the flow equivalent of the "filter unit".

Previously published data (5) established the value of K_f as 0.10 cc./cm.^{2}/min. /filter unit—on the finger pad. Application of this value to other skin areas yields reasonable data for the blood flows in these areas (7).

The relation of changes in blood content of the skin to variations in blood flow in the skin. Table 2 presents a series of simultaneous measurements on the same subject of changes in blood flow and in blood content of the finger pad. The constrictions in this series of observations and also in those referred to below were either "spontaneous" or those elicited by suitable stimuli (startle, pain, deep breath, etc); the dilatations were recoveries from previous constrictions. The durations of these changes varied from ten seconds to ten minutes. It is obvious that the blood content of the finger pad varies in the same direction as the changes in blood flow and that the relation between volume and flow is an approximately linear one. Some scatter in the values of the ratios occurs on successive vasomotor changes in the same individual as well as in averages from various individuals.

In a series of 48 observations on 7 subjects, the mean value of the ratio of volume change to flow change was 0.012; averages in these 7 subjects were distributed between 0.0102 and 0.0164. This means that a change in the rate of blood flow of 0.10 cc./cm.^{2}/min. in the finger pad will result in a corresponding change in the blood content of the pad approximately equal to 0.0012 cc./cm.^{2} of skin. This statement holds for either dilatation or constriction. The relatively minor deviations from the mean value of this ratio are related to such variables as the time required for a new volume equilibrium to be established following a change in flow and individual differences in the capacity of the vascular bed in the pad.

The relative constancy of the numerical value of the ratio of change in volume to change in flow in the finger pad may be employed to examine the possibility

of changes in venous tone occurring in the vascular reactions under observation. Thus, the relative constancy of the ratio in the vasomotor responses in the finger pad to such stimuli as noises, "psychic" stimuli, deep breaths, etc., suggests that in these instances changes in venous tone either do not accompany those on the arterial side or are approximately proportional to the changes in flow (they would then be masked by the latter). Large deviations, however, in the value of the ratio from the mean would imply the occurrence either of large or of disproportionate changes in venous tone.

Such effects may obtain during cutaneous vascular reactions to changes in temperature. They are readily illustrated by recording from the finger pad during the application of cold to the finger: The decrease in blood content of the pad skin during the initial constriction may be two to three times as great as that which would be expected to accompany the decrease in flow if the decrease in volume were in the same ratio to the decrease in flow as that which obtains during a vasoconstriction when the finger temperature is normal. This deviation from the predicted value of the ratio suggests that the decrease in volume resulting from arterial constriction and decreased inflow was reinforced by an unusually large increase in venous tone. This interpretation is in harmony with other observations described below.

TABLE 2

Relation of changes in blood content to changes in blood flow in the skin of the finger pad

CONTROL FLOW	CHANGE IN FLOW	CHANGE IN VOLUME	DURATION	$\frac{\text{CHANGE IN VOLUME}}{\text{CHANGE IN FLOW}}$
cc./cm.²/min.	*cc./cm.²/min.*	*cc./cm.²*	*seconds*	
0.078	+0.156	+0.00229	70	0.0140
0.105	+0.131	+0.00209	40	0.0158
0.079	+0.138	+0.00190	22	0.0137
0.217	−0.159	−0.00280	23	0.0143
0.050	+0.118	+0.00170	12	0.0144
0.052	+0.168	+0.00277	72	0.0165
0.222	−0.179	−0.00200	240	0.0112
0.071	+0.104	+0.00243	87	0.0234
0.374	−0.335	−0.00630	10	0.0188
0.356	−0.106	−0.00231	40	0.0218
0.160	0.159	0.00266	averages	0.0164

−: decrease +: increase.

The reactive dilatation which appears usually three to ten minutes after the application of cold is begun presents a hyperemia of the finger pad which is greater than can be accounted for by arterial dilatation. This is obvious in a qualitative sense from the sudden reddening of the skin and the engorgement of the capillaries. The increase in blood content may be three or more times that predicted from the increase in blood flow, and the blood content of the pad may remain far above the control level for varying periods of time, gradually

returning to the control level as the reaction to cold wears off. It is probable that the locus of this congestion is on the venous side, in the small veins and subpapillary plexus. Dilatation of these vessels by cold, plus their engorgement by the opening of arterio-venous anastomoses, would contribute to the development of this congestion which seems to be a readily reversible phenomenon if the chilling has not been too prolonged.

When we turn to skin regions other than the hands and feet and attempt to apply the procedure utilized above, we encounter certain complications. First, the low level of the resting blood flow in the skin of the trunk, arms and legs (7) makes very difficult the reliable demonstration of vasomotor reflexes acting on the arterial side of the circulation in these regions, particularly when the magnitude of the change in flow is only a small fraction of a flow already small (table 3). This difficulty does not hold for the relatively large changes in the

TABLE 3

Relation of changes in blood content to changes in blood flow in the skin of the forearm

CONTROL FLOW	CHANGE IN FLOW	CHANGE IN VOLUME	$\frac{\text{CHANGE IN VOLUME}}{\text{CHANGE IN FLOW}}$
cc./cm.²/min.	*cc./cm.²/min.*	*cc./cm.²*	
0.023	0.000	0.00049	
0.022	0.017	0.00054	0.032
0.015	0.002	0.00033	0.165
0.014	0.007	0.00034	0.049
0.025	0.0073	0.00054	0.074
0.016	0.005	0.00035	0.070
0.021	0.003	0.00106	0.350
0.021	0.0035	0.00123	0.350
Averages.. 0.019	0.0056	0.00061	0.156

All changes were decreases.

blood content of the skin (table 3). Second, the usual vasomotor discharges to the hands and feet affect neither the blood flow (8) nor the blood content (9) of the skin of the face and forehead. Third, our unpublished observations indicate that only a small fraction of the vasomotor discharges to the hand measurably affect either the blood flow or the blood content of forearm skin.

Table 3 illustrates the effects on forearm skin of vasomotor reflexes which acted here. These reflexes were elicited by psychic stimuli, application of cold to other regions of the body, etc. On comparing these reactions in the forearm skin with those in the finger pad, one notes that the change in blood flow in the latter is about eight times as great as the control level of flow in the forearm skin; the changes in the pad are correspondingly more striking. The *relative* change in flow (comparing constrictions only) was about twice as great in the pad as in the forearm (seventy per cent reduction in flow in the pad, thirty per cent in the forearm). Although this comparison is not based on records taken simultaneously from the finger pad and the forearm skin, the implications

are clear: vasomotor reflexes to the arteries of the forearm skin not only occur less frequently but also they are relatively less effective on flow when they do occur than those acting on the finger pad.

The changes in the blood content of the forearm skin due to vasomotor reflexes contrast sharply with the changes in flow; the ratio of the change in blood content to the change in blood flow is about thirteen times as great as that in the finger pad. Simultaneous variations in hand flow are not responsible for these changes in the blood content of the forearm skin since blocking the hand flow by an arm-cuff at the wrist does not eliminate this difference. It seems reasonable, therefore, to attribute these changes in blood content to changes in the tone of the small veins and venules of the skin.

TABLE 4

Effects of cold air on flow-volume relations in forearm skin

CONTROL FLOW	CHANGE IN FLOW	CHANGE IN VOLUME	$\frac{\text{CHANGE IN VOLUME}}{\text{CHANGE IN FLOW}}$	CHANGE IN TEMPERATURE	DURATION
cc./cm.²/min.	*cc./cm.²/min.*	*cc./cm.²*		°C.	*seconds*
0.017	0.010	0.0050	0.50	33 to 2	300
0.023	0.001	0.0009	0.90	31.5 to 8	40
0.022	0.008	0.0013	0.16	30 to 4	60
0.018	0.009	0.0016	0.18	33.4 to 12	90
0.027	0.012	0.0024	0.20	33 to 11	315
0.016	0.004	0.0016	0.40	34.5 to 15	120
0.030	0.006	0.0016	0.27		
0.016	0.001?	0.0017	?	33 to 15	55
0.021	0.006	0.0020	0.34	averages	

All changes were decreases.

This interpretation is reinforced by the results of an experiment in which a blast of cold air was directed against the forearm skin in the vicinity of application of the plethysmograph (table 4). The average change in blood content of the forearm skin was three times as great as that occurring during vasomotor reflexes (table 3), although the reduction in blood flow was approximately the same. Again, blocking of blood flow in the hand by an arm-cuff at the wrist did not affect this response to cold. It is also interesting to note that the venous constriction in forearm skin elicited by cold resulted in changes in blood content of the skin as great as those occurring in the finger pad during vasomotor reflexes although the change in flow in the forearm skin was only four per cent of the change occurring in the finger pad during vasomotor reflexes.

A similar study was made of the effects of blasts of cold air on the flow-volume relations in the skin of the forehead (table 5). The reduction in blood content was about the same as that in forearm skin but the reduction in blood flow was four times as great. Still, the average value of the ratio of change in blood content to change in flow is greatly in excess of that which would have been expected if the flow-volume relation in the forehead were the same as in the finger pad. (The absence of arterial vasoconstrictor reflexes in the skin of the forehead

does not allow one to test this point.) The application of the cold to the forehead elicited a sharp vasoconstrictor reflex in the finger but only a slowly developing decrease in flow and blood content in the forehead which roughly paralleled the fall in skin temperature. We interpret this to mean that both the arterial and venous constrictions in the forehead skin were due to the *direct* effect of the cold on these vessels.

DISCUSSION. The experimental data reported in this paper have implications which are significant for three important relations:

1. *The relation of change in blood content to change in blood flow as a measure of active venous participation in cutaneous vascular reactions.* When this relation does not remain relatively constant but shows large variations in its numerical value (this varies in the forearm over a range where the maximum value is ten times as great as the minimum—table 3) or where the numerical value of the

TABLE 5

Effects of cold air on flow-volume relations in forehead skin

CONTROL FLOW	CHANGE IN FLOW	CHANGE IN VOLUME	CHANGE IN VOLUME / CHANGE IN FLOW	CHANGE IN TEMPERATURE	DURATION
cc./cm.²/min.	*cc./cm.²/min.*	*cc./cm.²*		°C.	*seconds*
0.050	0.012	0.00095	0.079	31.2 to 12.4	30
0.047	0.017	0.00312	0.184	34 to 0	330
0.056	0.022	0.00214	0.097	35.5 to 6.7	270
0.058	0.005	0.00216	0.432	34.8 to 18.5	120
0.118	0.062	0.00217	0.035		
0.135	0.047	0.00330	0.070		
0.077	0.027	0.00230	0.148	averages	

All changes were decreases.

relation is excessively large (compare the finger pad—table 2—and the forearm—table 3), one may infer that changes in venous tone are occurring. This does not imply the converse, the absence of changes in venous tone, when the numerical value of the above relation remains relatively constant (as in the finger pad—table 2) and at the level which is characteristic of the finger pad. The evidence is then simply indecisive. This interpretation is in agreement with other plethysmographic evidence (1) (2).

It is of interest to compare the photoelectric estimations of changes in the blood content of forearm skin (table 3) with those which may be calculated from plethysmographic data (1). The latter indicated a change in forearm volume in the range, 0.3–7.3 cc./liter of forearm, in response to such stimuli as pinching and mental arithmetic. Assuming that these changes occurred only in the cutaneous vessels and re-calculating the changes in volume in terms of the surface area of the skin, the values become 0.00054–0.0142 cc./cm² skin. (The calculations assume that 13.5 per cent of the forearm volume is skin (1) and that each cubic centimeter of skin has an area of about 3.9 cm²/(10).) The changes in blood content of forearm skin reported in tables 3 and 4 are con-

siderably less than those calculated from mechanical plethysmograms of the forearm. We are inclined to the opinion that the discrepancy in the two groups of data arises out of the inclusion of main vessels, particularly the venous drainage, in the forearm plethysmogram and also from the assumption that the changes in the latter are to be referred wholly to the skin.

2. *The estimation of the quantity of blood in the minute vessels of the skin.* One may attempt to approximate the magnitude of the vascular bed in the skin from the following calculations: If we assume that the constriction in forearm skin to cold is very nearly maximal and that the residual blood content of the skin is only a small fraction of the quantity which is normally present, the change in blood content of the skin in table 4 approximates the amount of blood which is present in the minute vessels of the skin. This quantity is about 20 cc./M^2 of skin.

That this quantity is reasonably correct may be argued from analogy with similar data on the intestinal loop (11). Schleier's data (table 4—reference 11) gives a ratio of blood content of the minute vessels (small arteries with a radius of 0.25 mm. to small veins with a radius of 0.32 mm.) to blood flow of 5.33 cc./70 cc./min. or 0.076. Similarly, in the forearm skin, this ratio has the numerical value of 0.095 (0.0020/0.021—table 4). We do not know, of course, whether we have made strictly comparable calculations in the two series of data, but the analogy is striking.

The implications in these calculations receive some additional support from estimates of the blood content of the venules which may be made from the data of Wetzel and Zotterman (12). The figures are: 2.5 cc./M^2 of forearm skin and 18.6 cc./M^2 of cheek skin. Since the measurements from which these estimates are derived were made at room temperatures of 17–21°C, it is probable that the venous vessels were considerably constricted during the observations of Wetzel and Zotterman. Correction of these figures by the inclusion of larger vessels (following the analogy on the intestinal loop (11)) brings them into better agreement with the data in this paper but they are still considerably lower.

It is to be emphasized that the figure for the blood content of the skin as calculated above applies only to the minute vessels. Since they would contain only 40 cc. of blood in an individual with a surface area of $2M^2$ (assuming an average skin flow of 220 cc./M^2—(7)), they cannot be considered as an important blood depot. This conclusion does not imply that the currently taught concept of the skin as an important blood depot is in error but it does suggest that this function is served principally by the larger vessels. A similar conclusion is reached from the examination of Schleier's data on the intestinal loop (11), and also from an exploration of the distribution of blood in the vascular system of the dog by the method of radioactive isotopes (13) which showed that about seventeen per cent of the total blood is contained within the minute vessels. In skeletal muscle, these vessels hold about 0.02 cc./gram of tissue. (No values were given for skin but the statement was made that its blood content was very low.) Re-expression of the cutaneous values given above yields a unit value of 0.008 cc./gram of skin. There can be little doubt therefore that the photo-

electric estimation of the blood content of the minute vessels of the skin is approximately correct.

3. *The relation of skin color to skin blood flow.* The relation of the skin color to blood in the subpapillary plexus (12) and the data in this paper indicate that the quantity of blood in the skin and the changes in the blood content of the skin can be quite disproportionate to the level of blood flow and to the changes in flow and are indeed a very unreliable guide to the latter. Several of our unpublished experiences with the effects of hemorrhage on the skin circulation in man emphasize this point; during the so-called "reaction" which is exhibited by a small fraction of blood donors and which is characterized by a fall in blood pressure, peripheral dilatation and symptoms of cerebral hypoxia, we have seen an increase in cutaneous blood flow and pallor develop simultaneously. The simultaneous pallor must then represent an increase in venous tone. Similar divergence of skin blood content from skin blood flow is illustrated in the engorgement of the skin without an increase in flow in some subjects to whom estrogen has been administered (13).

SUMMARY

1. The estimation of the blood content and the blood flow in the skin by use of the photoelectric plethysmograph is described and analyzed.

2. It is demonstrated that during spontaneous or induced constrictions in the finger pad, blood content and blood flow vary in the same direction and in an approximately linear fashion. Calculations show that a change in rate of flow of 0.10 cc./cm.^{2}/min. is accompanied by a corresponding change in blood content of 0.0012 cc./cm.^{2} of skin. Evidence is presented to demonstrate that the relative value of the ratio of volume change to flow change may be employed to study the possibility of changes in venous tone occurring during vascular reactions. That is, large deviations in the value of this ratio from the mean would imply large or disproportionate changes in tone of the small veins.

3. Vasomotor reflexes in forearm skin are relatively less effective on flow than those occurring in the finger pad. The ratio of change in blood content to change in blood flow is about thirteen times as great as in the finger pad. It is suggested that these changes in blood content are due to changes in tone of small veins and venules in the skin.

4. Studies on forehead skin show a ratio which is greatly in excess of that demonstrated in the finger pad. Absence of arterial vasoconstrictor reflexes in the forehead skin suggest that constrictions occurring in this region (as a result of exposure to low temperatures) are due to direct effects of the cold on the small arteries and veins (arterioles and venules).

5. The estimation of the quantity of blood in the minute vessels of the skin is discussed. Calculations from data presented show that the total quantity of blood normally present in these vessels is relatively small.

6. The unreliability of dependence upon the intensity of skin color as determined by blood content of the subpapillary venous plexus in the estimation of the skin flow is discussed.

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STUDIES ON THE RECURRENCE OF DECOMPRESSION SICKNESS ON RE-ASCENT TO HIGH ALTITUDES

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Decompression sickness, which is sometimes seen after rapid ascent from deep sea diving or after ascent from sea level to high altitudes, may become manifest in a variety of symptom complexes. Bends, the most common form of these disturbances, is characterized by pain referred to the bones, joints and muscles of the extremities. The pain may be mild and even disappear while the subject remains at reduced pressure. Frequently, however, the pain is progressive, leading in time to severe distress and eventuating in collapse. In these latter cases bends can become a serious hazard in flights at high altitudes.

Disturbances which are induced less frequently by reduced barometric pressure include rashes, chokes, headaches, vomiting, scotomata, and paresthesias and paralyses which may involve any portion of the body. Except for chokes, these are relatively rare. The term, chokes, aptly describes the rapid development of inspiratory dyspnea, tachypnea, and a choking sensation which quickly leads to a subjective feeling of impending disaster.

On recompression, the variegated symptoms of decompression sickness usually disappear quickly during or shortly after recompression is accomplished. Muscle soreness and mild joint pain may remain as a residue from an experience of bends. Chokes is frequently followed by episodes of non-productive coughing which give way within an hour to a severe generalized throbbing headache, and this may persist for many hours. Symptoms which indicate neurological involvements such as scotomata and paralyses may sometimes be progressive, leading to a shock-like condition which has culminated in several instances in death.

The ever-increasing air traffic at altitudes at which decompression sickness may occur, and the possibility that these symptoms may become much more common after the war, have indicated the need for further investigations into the nature of decompression sickness. An opportunity for such studies was utilized during routine training of Army Air Force aircrew members who, for purposes of selection and training, participated in simulated "flights" in the decompression chamber.

About twenty per cent of these subjects experienced some form of decompression sickness. Statistical analyses of the experiences of some altitude chambers which indicate the relationship of age, weight, height, time of day, and other factors have already been published (1, 2, 3). Some cases of clinical interest have also been reported (4, 5, 6).

In the present study it was felt that some insight into the mechanism of

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decompression sickness might be obtained by re-exposing personnel to a second flight shortly after recovery from decompression sickness. In this way it was hoped that data might be obtained concerning the effect of several factors upon the likelihood of recurrence of decompression sickness on the second flight.

Studies were conducted on flying personnel and on some members of the regular Altitude Training Unit crew in the decompression chamber at Davis Monthan Field, Tucson, Arizona (altitude 2500 ft.) in May and June of 1943. The usual flight began with a trial ascent to 5000 feet and return to ground level. The chamber then ascended at about 3000 feet per minute to 20,000 feet and all passengers were subjected to an anoxia test. This test was performed by disconnecting each passenger from the oxygen supply for 5 to 10 minutes, so that each one might personally experience mild anoxia. The chamber then ascended to 38,000 feet (155 mm. Hg) and remained at this altitude for periods up to two hours. Passengers reporting the development of bends or other symptoms of decompression sickness were, after variable periods of time, removed from the chamber to the lock and returned to ground level barometric pressure. After a rest period at ground level, some of these latter subjects were returned to the test altitude of 38,000 feet to determine whether symptoms of decompression sickness would quickly recur.

Only those subjects who appeared to have recovered completely from the effect of the first flight were reflighted. On the reflight the subjects remained at 38,000 feet for five minutes. It was sometimes necessary because of the development of severe pain to lower the chamber before the five minute period had elapsed.

During each flight, records were made of the rate of ascent, time at altitude before onset of symptoms, and the altitude at which pain disappeared during descent. *Time of onset* of bends is the period spent at 38,000 feet before the subject first noted the pain. *Duration* of the pain was taken as the time from the onset of the condition until the chamber began the descent from 38,000 feet. Usually the pain lasted for a short while longer until the chamber reached 30 to 20 thousand feet. In a like manner, *rest period* was calculated as the time period beginning with the moment the chamber left 38,000 feet on the descent, until the subject was returned to 38,000 feet.

The term, *recurrence*, is used to indicate that the pain reappeared in the *same* anatomical site which had been involved on the original flight in the first five minutes at 38,000 feet on the reflight. *Time of recurrence* is given as the time of appearance of symptoms on the reflight.

Results. One hundred and fifty-three subjects who had experienced one or more symptoms of decompression sickness on the regular training flight participated in a second flight within 30 hours. Of these, 149 had bends on the first flight, 17 had chokes, 2 had rashes, 4 had visual disturbances, 2 reported headaches, 3 had profuse sweating, 3 had faintness, and 2 had nausea. Analyses of these symptoms with reference to the several variables mentioned are given below.

1. *Effect of the duration of the rest period.* Bends recurred in an anatomical

site involved in the first flight in 63 of 149 subjects taking the reflight. Study of the data indicated that the duration of the rest period was the most significant factor in the probability of recurrence on the reflight (table 1). Thus all five cases with rest periods up to 20 minutes (time for descent and immediate re-ascent) had recurrences on the reflight. With rest periods ranging from 21 to 180 minutes, bends recurred in approximately half of the 117 subjects tested. No subject with a rest period longer than 180 minutes had a recurrence of bends during the five minutes of the reflight.

2. *Comparison of the times of onset of bends.* In the 63 subjects with recurrence of bends, the time of onset of bends on the first flight was compared with the time of onset on the reflight. The average time of onset on the first flight was 28 minutes, ranging from 0 to 83 minutes, while the time for recurrence in the same subjects averaged 2 minutes on the reflight. Comparison of the two groups is given in table 2.

TABLE 1

Effect of the rest period on recurrence of bends

REST PERIOD	TOTAL CASES	RECURRENCES	RECURRENCES
minutes		*number*	%
0–20	5	5	100
21–40	23	11	48
41–60	29	15	52
61–80	24	12	50
81–120	28	15	54
121–180	13	5	46
181–300	4	0	0
301–1800	23	0	0
Totals..............	149	63	

The striking difference between the time of onset of bends in the two flights indicates that the experience of bends on the first flight predisposes the subject to early onset on an immediately succeeding (within 180 min.) flight.

3. *Effect of the rest period upon the time of recurrence of bends.* The duration of the rest period had a highly significant influence not only upon the likelihood of recurrence of bends, but it also appeared to affect the time of return of bends on the reflight. This is shown in the direct correlation between the duration of the rest period and the average time of recurrence in each group (table 3).

This relationship is also evident when it is seen that in subjects with shorter rest periods, bends tended to recur more frequently during re-ascent, before the chamber had reached the test altitude of 38,000 feet during the reflight. Fourteen cases with recurrence of bends during the re-ascent are also analyzed with reference to the rest period in table 3. It is evident that short rest periods predispose to an almost explosive recurrence of symptoms during the re-ascent.

4. *Bends duration and time of recurrence.* The rapid recurrence of bends during the reflight was related not only to the duration of the rest period, but was

also correlated with the duration of bends during the first flight. This is shown by the fact that the average time before recurrence on the reflight is less in those subjects with longer durations of bends. This relationship can also be seen in the analysis of data obtained from the 14 subjects in whom bends recurred during the ascent on the reflight, before the altitude chamber reached 38,000

TABLE 2

Time of onset of bends

TIME OF ONSET	FIRST FLIGHT	SECOND FLIGHT
minutes	*subjects*	*subjects*
0*	5	14
1	3	9
2	0	16
3	0	10
4	0	6
5	3	8
6–10	8	—
11–25	11	—
26–50	25	—
51–75	6	—
76–83	2	—
Totals....................	63	63

* Indicates onset during ascent, before chamber reached 38,000 feet.

TABLE 3

Relationship between rest period and rapid recurrence

REST PERIOD	CASES WITH RECURRENCE	AVERAGE TIME OF RECURRENCE	SUBJECTS WITH RECURRENCES DURING REASCENT	RECURRENCES DURING REASCENT IN EACH GROUP
minutes		*minutes*		%
0–20	5	1.0	3	60
21–40	11	1.6	4	36
41–60	15	1.8	5	33
61–120	27	2.6	2	8
121–180	5	2.4	0	0
Totals..........	63		14	

feet. Thus, only 3 out of 30 subjects with bends for 8 minutes or less had a recurrence during the re-ascent, while 11 of 33 with bends for more than 9 minutes had a recurrence during re-ascent.

These results clearly indicate that subjects with long durations of bends are more likely to have recurrences during re-ascent, or immediately after reaching 38,000 feet, than those with shorter durations of bends (table 4).

5. *Grade of pain of bends.* The pain experienced in bends ranged from a sharp, throbbing, highly localized pain to a dull aching generalized pain affect-

ing a region or an entire limb. Each subject was asked to identify the severity of pain according to the following classification:

Grade 1. Mild transitory pain
Grade 2. Persisting mild or moderate pain
Grade 3. Severe pain which did not permit concentration on any task
Grade 4. Excruciating pain demanding immediate descent.

Grade 4 pain was usually associated with general symptoms such as profuse sweating, faintness or nausea. Pain which passed through more than one grade was recorded at its most severe grade. Thus mild pain which persisted for 30

TABLE 4

Effect of duration of bends on the time of recurrence

DURATION OF BENDS	SUBJECTS WITH RECURRENCE	AVERAGE TIME OF RECURRENCE	SUBJECTS WITH RECURRENCE DURING RE-ASCENT
minutes		*minutes*	
1–3	13	2.7	1
4–8	17	2.5	2
9–25	20	1.6	8
26–50	10	1.6	3
50–100	3	1.0	0
Totals..............	63		14

TABLE 5

Grade of bends and likelihood of recurrence

GRADE OF BENDS FIRST FLIGHT	NUMBER OF SUBJECTS IN EACH GROUP	NUMBER WITH RECURRENCE	NUMBER WITH SAME GRADE OF BENDS ON BOTH FLIGHTS
1	10	1	1
2	42	21	13
3	60	35	15
4	14	6	3
Totals..............	126	63	32

minutes and which became excruciating in character just before descent, was recorded as grade 4 pain, duration 30 minutes.

All cases with rest periods up to 300 minutes were analyzed to determine if there might be a relationship between the intensity of pain on the first flight, and the likelihood of recurrence on the reflight. It was seen that while grade 1 bends recurred in only one of ten subjects, the other three grades of bends recurred in approximately half of the subjects. It was also noted that when bends did recur, about half of the subjects reported that the pain was approximately of similar grade in both flights. Data are given in table 5.

6. *Occurrence of new bends during reflight.* Of the 149 subjects with bends during the first flight, 11 (7 per cent) reported the occurrence of bends during the

five minutes of the reflight in an anatomical site not involved during the first flight (new bends). These data are presented in table 6.

These results do not indicate any difference between the two groups. It is obvious that on occasion new bends may occur during the reflight in a new anatomical site, and this occurrence need not necessarily be related to the *recurrence* of bends in other sites.

7. *Chokes.* Symptoms of chokes tended not to recur on the reflight. Thus, of 17 cases which were reflighted, one reported a recurrence of symptoms. Bends were also reported by 13 of these subjects, and of these, bends recurred in 8 (table 7).

TABLE 6

Occurrence of new bends on the reflight

	TOTAL NO. SUBJECTS	NO. WITH NEW BENDS	NEW BENDS
			%
Subjects with no recurrence	86	7	8
Subjects with recurrence	63	4	6
Totals	149	11	

TABLE 7

Comparison of recurrence of bends and chokes

REST PERIOD	NUMBER OF SUBJECTS	SUBJECTS WITH RECURRENCE OF CHOKES	SUBJECTS WHO ALSO HAD BENDS	SUBJECTS IN WHOM BENDS RECURRED
minutes				
0–20	4	0	3	3
21–80	11	1	8	5
81–300	2	0	2	0
Totals	17	1	13	8

8. *Recurrence of symptoms other than bends or chokes.* The relative rarity of symptoms of decompression sickness other than bends and chokes made it difficult to establish whether or not there was a tendency for these symptoms to recur. Results on the few subjects that were tested are given below.

Both subjects with rash who were studied had a recurrence of the rash on the reflight.

Blurring of vision with scintillating scotomata may occur not only while the subject is at high altitudes, but may ensue during descent or shortly after reaching ground level (5). These visual disturbances usually regress rapidly, and disappear completely within an hour or two. Four subjects who had experienced blurring of vision on the first flight were returned to 38,000 feet after 62, 68, 113 and 118 minutes of rest, respectively. In none of these was there a recurrence of the visual disturbance.

A moderate or severe headache frequently follows the visual disturbance. In two cases the headache persisted during the rest period, and appeared to be aggravated during the reflight.

Other symptoms such as profuse sweating, faintness or nausea did not recur in 5 subjects.

DISCUSSION. Decompression sickness is now generally believed to be due to the formation and persistence of bubbles in sensitive regions (7). It has been suggested that these bubbles are present in the tendon sheath, and that they produce symptoms by stretching and tearing (8). Inspection of our data leads to the conclusion that most of the phenomena described can be easily explained in physiological terms and through the use of the physical laws pertaining to the behavior of gases.

The gases dissolved in the tissues are normally in equilibrium with the gases in the pulmonary alveoli, except that because of metabolic activity the tissue oxygen tension is lower than that in the alveoli and the arterial blood; and the tissue carbon dioxide tension is somewhat higher than that of the venous blood and the alveoli. Thus the tissue oxygen tension would be less than 100 mm. Hg, and the tissue carbon dioxide tension would be somewhat more than 40 mm. Hg. The tissue nitrogen tension is in equilibrium with the alveolar nitrogen tension, being approximately 580 mm. Hg at ground level.

On decompression to 30,000 feet or above, where the total barometric pressure is approximately 200 mm. Hg or less, the nitrogen in the tissues becomes supersaturated, and the gradient is such that the nitrogen passes from the tissues where the nitrogen tension is high, via the blood stream, to the alveoli where the nitrogen tension is low. If this transport is inadequate, as in regions with poor circulation, the nitrogen will tend to come out of solution and form bubbles. The phenomenon of Reynold's cavitation, which occurs with sudden pressure changes, as may occur during muscular contraction, may contribute to the process of bubble formation (9).

After formation during or after ascent on the first flight, such bubbles would continue to grow at high altitudes as long as the partial pressure of the nitrogen in the tissues was greater than that in the adjacent bubbles. Recompression would decrease the size of the bubbles, and increase the tendency for the gaseous constituents to return to solution. With short rest periods, such a bubble would not have time to be completely re-dissolved, and with re-ascent it would expand quickly with the falling pressure and cause an almost immediate recurrence of the symptoms of bends. However, if sufficient time was allowed before re-ascent, and this is of the order of 3 hours according to our results, the bubble would be dissipated completely, and the process of formation and growth of the bubble must begin anew.

The recurrence of bends in the same anatomical site after short rest periods suggests that if bubbles are present in such subjects, they must be in sites from which they cannot migrate, such as in the extravascular tissues.

If bubbles were intravascular and producing symptoms of bends as a result of their embolus-like action, they would shrink with increased barometric pressure

on descent, and thus might be freed to pass on to the lungs and occasionally produce symptoms of paradoxical chokes or neurological disturbances (5). On re-ascent, such bends would not be expected to occur in the previously affected site if all the bubbles in sensitive locations were washed away. The results reported in this study could thus be interpreted as showing that approximately half of those subjects with bends had the bends-producing bubbles in the extravascular tissues. The remaining half of the subjects had bends due to bubbles within the vascular system, since with rest periods between 21 and 180 minutes, half of the subjects had recurrences and the remainder did not.

Our results showing that the time at altitude on the reflight before recurrence of bends is shortened in subjects with long durations of bends on the first flight, can be interpreted to indicate that after bubbles form and cause bends, they will continue to grow in size as long as the partial pressure of the nitrogen in the tissues is greater than the partial pressure of the nitrogen in the adjacent bubble. If the bubble at the end of the first flight is large, it is to be expected that a larger residue would remain, all other conditions being equal, when the subject returns to high altitude. Therefore, symptoms of bends would be expected to recur more rapidly in subjects with longer duration of bends on the first flight.

The failure, except in 1 out of 10 cases, of mild transient bends to recur is easily explained by the assumption that the original bubble was either small and therefore quickly dissipated, or that it was in a relatively insensitive site. If the original bubble were small, it would be expected that the residue would also be minimal.

The more severe grades of bends, 2, 3 and 4, all behaved similarly in that approximately 50 per cent of the subjects in each group reported recurrences. This incidence of recurrence cannot be explained on the basis of bubble size since it would be expected that the more severe bends would be related to larger bubbles. In such an event, it would be expected that a greater incidence of recurrence would occur with grades 3 and 4 than in 2. It is apparently necessary to interpret these findings by suggesting that the degree of pain is due more to the sensitivity of the site affected, than to the volume of the causative agent, the bubble. This is in accord with the work of Ivy and his associates (10) who showed that bubbles and pockets of gas may exist in the tissues without reference to the presence or grade of pain referred to the site.

This latter point is supported by the fact that the grade of pain on the second flight was usually similar in character to that experienced in the first flight. This would suggest that the bubbles causing the pain remained in the same site during the descent and rest period, and on expansion with re-ascent they stimulated essentially the same pain receptors which were involved in the first flight.

The incidence of the occurrence of bends on the reflight in anatomical sites not involved during the first flight is of the order of occurrence of bends during the first flight. It is likely that the mechanism responsible for these bends is identical with that involved in the formation and growth of bubbles and the production of bends in the first flight. It is even possible that a small proportion of the cases included in the *recurrence* data might also be due to new bubble formation rather than to the expansion of previously formed bubbles.

Several factors which were analyzed appeared to have no clear relationship to the likelihood of recurrence of bends. These included the rate of ascent, time of onset of bends on the first flight, anatomical site or the number of sites involved, and the altitude of disappearance of pain during descent.

The failure of chokes to recur suggests that the bubbles which might be responsible for this condition are probably intravascular. On recompression, their reduced size would permit them to move to other locations in the vascular system to produce paradoxical chokes (5), or perhaps their gaseous constituents might be lost in the alveolar spaces. If these bubbles were capable of passing through the pulmonary blood vessels, they could go on to produce systemic disturbances such as are occasionally seen to occur during or after the return to ground level. Thus, if these bubbles lodged in the central nervous system, they could easily produce scintillating scotomata, vomiting, collapse, and other disturbances depending upon the neurological site involved (5).

SUMMARY

1. Passengers in the low pressure chamber who experienced symptoms of decompression sickness were, after variable periods of rest at ground level, returned to 38,000 feet simulated altitude to determine the likelihood of immediate recurrence of symptoms.

2. When the subject was permitted to remain for 180 minutes or more at ground level between flights, symptoms did not recur. With shorter rest periods, half the subjects with bends had a recurrence of this condition within the first 5 minutes at altitude.

3. When the duration of bends on the first flight was increased, the time before recurrence on the reflight was lessened.

4. Mild transient bends tended not to recur. The more severe grades of bends recurred in half the subjects tested.

5. Chokes tended not to recur, even in subjects with recurrence of bends.

6. The phenomena observed are discussed in terms of the concept that decompression sickness is due to the presence of bubbles in the tissues and blood vessels.

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THE EFFECT OF OXYGEN, ALTITUDE AND EXERCISE ON BREATH-HOLDING TIME

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Until recently, the duration of voluntary apnea had been recorded in a variety of situations such as mountain climbing expeditions (1, 2), in certain diseases, particularly those of the pulmonary and cardiovascular systems (3), in evaluating physical fitness and anxiety states (4), and for instructional purposes (5). The second World War renewed interest in breath-holding and it was again tested for its utility in the evaluation of physical stamina (6), in the study of the effects of high altitude in the decompression chamber (7, 8, 9) and in testing for neurocirculatory asthenia (10, 11).

The second World War renewed interest in breath-holding since it appeared to have survival value in emergency situations in which oxygen tensions in the inspired air is low, or in the presence of fire or noxious gases. The studies outlined in this paper were directed at an evaluation of the utility of breath-holding in escape from aircraft at high altitudes. In order to analyze factors which might affect the time of the hold, we determined the effects of altitude, of certain oxygen tensions, of hyperventilation and of a short burst of work equivalent to an attempt to leave an airplane in flight.

METHODS. Fifteen hundred and eighty-one determinations of breath-holding time were made on 80 volunteers at the Altitude Training Unit, Kingman Army Air Field, Arizona (altitude 3400 ft.), and at the Aero Medical Laboratory, Wright Field, Dayton, Ohio (altitude 500 ft.) during November-December 1944. The subjects were told to take a single deep inspiration and to hold it as long as possible, but to exhale before extreme pain and discomfort occurred. When the subjects first attempted to hold the breath, unfamiliarity with the maneuver caused them to terminate the test after about 30 seconds. Confidence developed after a few trials, and the subjects became remarkably consistent in successive measurements. In an early series of 341 consecutive determinations while the subjects were at rest at ground level breathing air, the average hold was 70 seconds, ranging from 34 to 125 seconds. Since the responses to various procedures were proportional to the hold at rest while at ground level and breathing air, our results are expressed as percentages of this average hold of 70 seconds.

RESULTS. 1. *The variability of the breath-holding time.* The apparent great variability of the breath-holding time seen from subject to subject, and even in the same subject at various times has unquestionably deterred many workers from using this technique for the study of problems in respiration, work, fatigue and the like. Analysis of our data indicated that the hold is quite constant when only a few minutes intervenes between readings. There may be shifts of the

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base line. Usually an increase in breath-holding time occurs with repetition of the test. Nevertheless, randomization of the control readings makes it clear that useful data may be obtained. This is illustrated in the results of a typical experiment on a volunteer lying at rest on a cot at ground level, breathing air (table 1).

By taking 2 deep breaths and holding the second, 5 subjects increased their breath-holding time an average of 16 per cent. This was increased but slightly by taking three deep breaths and holding the third, or taking 5 deep breaths and holding the fifth. After hyperventilating at ground level for 30 seconds the hold was increased 45 per cent.

In 28 resting subjects who took either a single deep breath of oxygen, or breathed oxygen for one or two minutes, the breath-holding time was increased an average of 80 per cent over the control values.

TABLE 1

TIME P.M.	CONTROL HOLD	HYPERVENTILATED FOR 30 SECONDS WHILE	
		breathing air, hold	breathing oxygen, hold
	seconds	*seconds*	*seconds*
2:30	53		
2:33	59		
2:37	63		
2:42		88	
2:49			127
2:55	58		
3:01			142
3:09		93	
3:14	64		
3:20		97	
3:27			160
3:32	77		
Average............	62	93	143

2. *The effect of altitude.* The hold decreased progressively with increasing altitude when the subject breathed ambient air (fig. 1).

The breath-holding time increased above normal when the subject breathed pure oxygen at altitudes below 25,000 feet; above that altitude the hold was less than normal and decreased progressively with decrease in atmospheric pressure (fig. 1).

The demand oxygen regulator (type A-14) used by the Army Air Forces was designed to deliver a mixture of oxygen and air calculated to maintain the normal ground level alveolar oxygen tension. When the regulator delivered this mixture, no significant change in the hold was noted until altitudes above 23,000 feet were reached. Above that altitude, the breath-holding time decreased with decreasing barometric pressure (fig. 1).

3. *The effect of exercise.* The effect on the hold of the moderate exercise in-

volved in 10 deep knee bends performed in 15 seconds was determined at several altitudes in 105 trials. The subjects took 10 deep knee bends, sat down, took a deep breath and held it as long as possible. This exercise was found to be equivalent to the performance on the bicycle ergometer of about 2250 foot pounds of work in 15 seconds.

The average breath-holding time after 10 knee bends was found to be about 30 seconds (40 per cent of control) at altitudes above 10,000 feet and did not appear to change significantly with greater altitudes up to 28,000 feet. Above 28,000 feet the breath-holding time after exercise appeared to be further reduced.

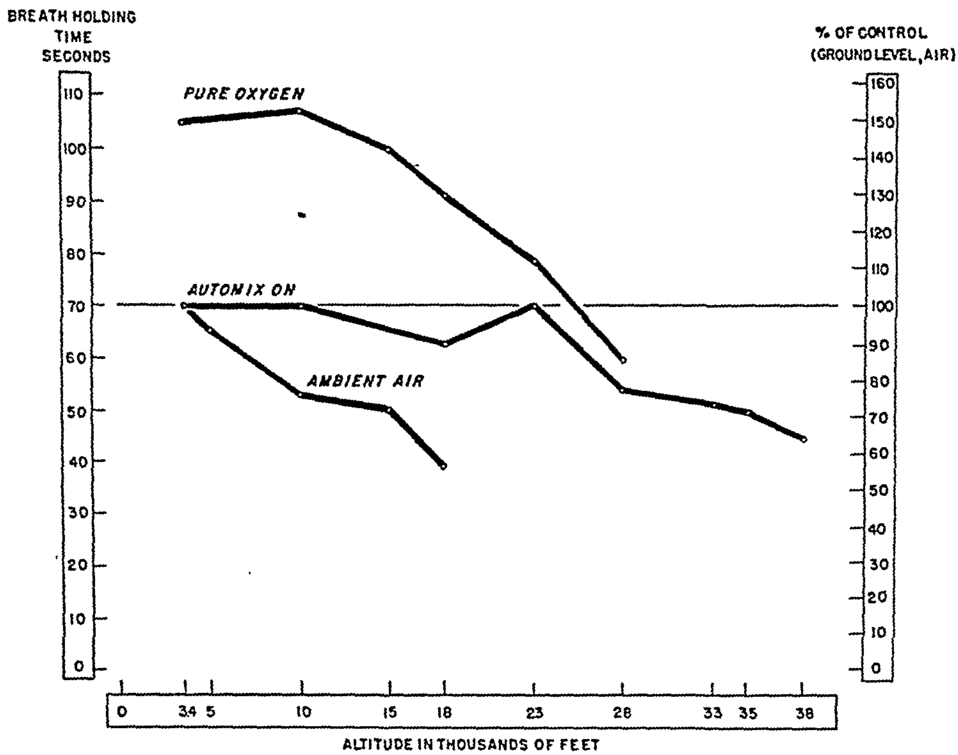

Fig. 1. Effect of altitude on breath-holding time. Upper curve: obtained with subjects breathing pure oxygen prior to hold. Middle curve: obtained with subjects breathing "automixed" air and oxygen from a demand regulator (type A-14) as described in the text. Lower curve: obtained with subjects breathing ambient air. Each point represents an average of at least 7 determinations.

There was no significant difference between the group breathing pure oxygen during the exercise and the group using the oxygen-air mixture furnished by the regulator (fig. 2).

Short bursts of work lasting 15 seconds on the bicycle ergometer in 56 tests on 4 subjects at ground level showed clearly that the breath-holding time is reduced in proportion to the degree of exertion (fig. 3). After exercise, recovery of the resting ability to hold the breath begins at once on resumption of respiration and appears to be complete within 5 minutes.

The performance of 10 knee bends in 15 seconds caused a doubling in minute

respiratory volume when no attempt was made to hold the breath in 10 subjects. The debit in respiratory ventilation volume resulting from breath-holding was quickly repaid by spontaneous hyperventilation occurring immediately after resumption of breathing. Most of the debit was repaid within the first minute after respiration was resumed. Substitution of 100 per cent oxygen for air did not affect the rate or degree of this recovery.

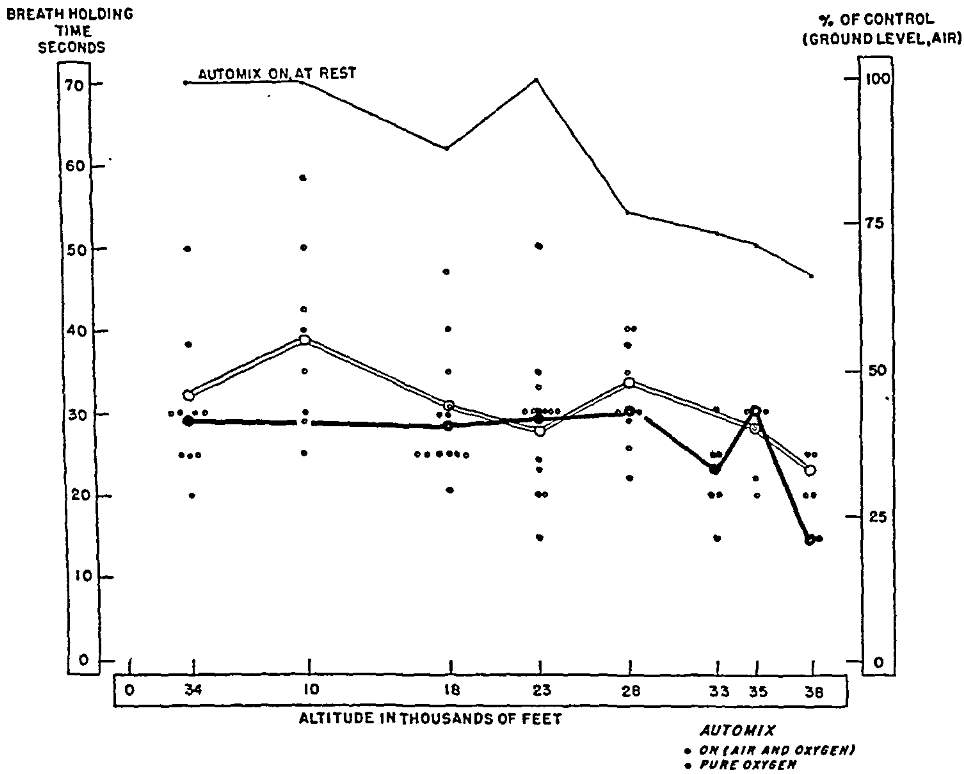

Fig. 2. The effect of 10 knee bends on breath-holding at altitudes up to 38,000 feet. Upper curve: this is the middle curve of figure 1, used as a line of reference. Solid line: breath-holding time at various altitudes while breathing oxygen-air mixture as described in text. Solid circles are individual determinations. Double line: average breath-holding times obtained when the subject breathed pure oxygen. Open circles are individual determinations.

4. *Escape from "spinning aircraft"*. Six subjects were tested for breath-holding ability in 16 trials while attempting to crawl through a hatch against the increased force of gravity, induced in the spinning centrifuge. They wore summer or winter flying clothes with a back-pack parachute weighing about 20 pounds. With 1.6 to 2.4 *g* (resultant between the lateral force induced by the spinning centrifuge and that of the downward pull of gravity) they were able to leave the hatch in 15 to 25 seconds. Breath-holding was begun at the beginning of the attempt to escape, and the hold under these conditions was found to be 18 to 28 seconds, averaging 23 seconds. The time to get through the hatch averaged 17 seconds. Thus the average breath-holding time after leaving the "aircraft" was 6 seconds.

5. *The effect on the oxygen saturation of the blood.* An indirect measure of oxygen saturation of the blood while holding the breath at several altitudes was obtained by means of the Millikan oximeter (12). The recording unit was placed on the ear and calibrated at 96 per cent when the subject breathed air at ground level. At 5, 10 and 15 thousand feet, the oximeter reading indicated a slight increase in oxygen saturation within a few seconds after the hold was commenced. As the hold continued the reading declined progressively until breathing was again resumed. Within a few seconds after respiration had begun again the oximeter reading fell sharply, and then began to return to the control reading, reaching this value in 20 to 30 seconds. Average data are indicated in table 2.

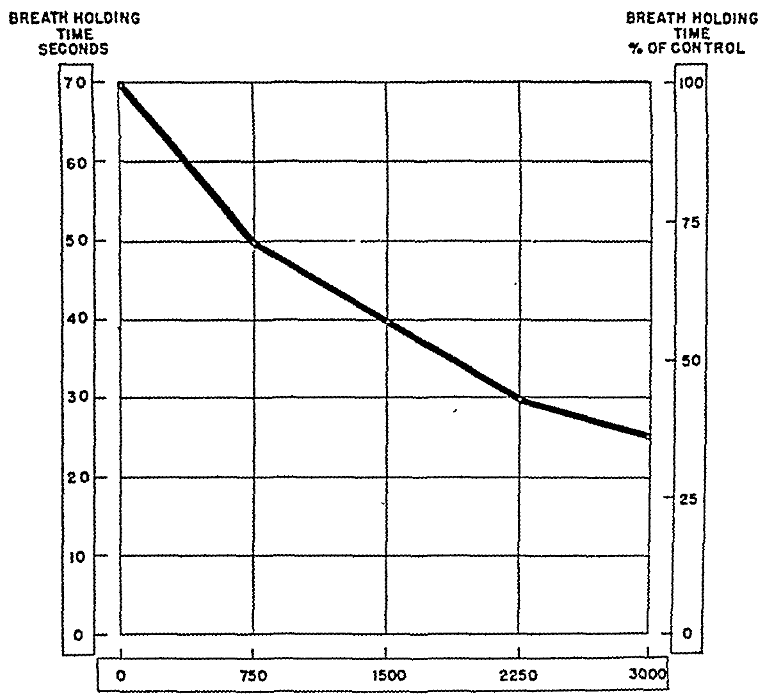

Fig. 3. Effect of a sudden burst (15 sec.) of pedaling on the bicycle ergometer on the ability to hold the breath immediately after exercise. Each point is an average of at least 7 determinations.

When breathing 100 per cent oxygen at ground level no change in the oximeter reading was observed in four tests even though the subject held his breath for 140 to 160 seconds. At 15,000 feet there was a fall of only 2 points in subjects breathing 100 per cent O_2 and holding the breath for 130 seconds.

The results suggest that at the onset of breath-holding, the increased intrathoracic pressure resulting from the Valsalva maneuver increases the effective pressure in the lungs and thus causes a greater oxygenation of the blood. Conversely, at the end of the hold, the sudden release of intrathoracic pressure results in a similar fashion in a decreased oxygen tension of the blood. Such a mechanism would tend to raise the blood oxygen saturation somewhat during breath-holding. The increasing tonus of the diaphram and the thoracic wall during

breath-holding probably plays a large part in the production of the discomfort which finally causes an end of the hold.

In 10 tests, exercise (10 knee bends) had little or no effect on the oxygen saturation readings during the subsequent breath-holding period. On resumption of breathing a transient sharp fall of 8 to 10 points in the oximeter reading was usually observed. No difference was seen in the groups breathing air or oxygen.

Discussion. The effect of hyperventilation with air in increasing the hold probably may be due to the loss of blood carbon dioxide. The increase in the hold when the subjects were breathing pure oxygen can be ascribed only to the increase in oxygen tension in the alveoli and the blood. The fact that breathing of pure oxygen for 2 minutes was not of much more value than a single deep breath of oxygen re-emphasizes the fact that the ability of the body to store oxygen in the tissues is severely limited. This is also in accord with the findings of Ferris and his associates (13) that breath-holding time varies directly with the oxygen content of the blood, and that under conditions of rest, the factor precipitating the breaking point is dependent on O_2 lack. Hyperventilation while breathing pure oxygen combines the individual beneficial effects of increased oxygen tension and of lowered alveolar carbon dioxide tension. Our results suggest that under conditions of exercise, CO_2 excess precipitates the end of the breath-holding maneuver.

TABLE 2

Oximeter readings during breath-holding at various altitudes

NO. TRIALS	ALTITUDE	READINGS AT 10 SECOND INTERVALS DURING BREATH-HOLDING													AFTER RESUMPTION OF BREATHING			
															Minimal reading	Seconds		
		Control	10	20	30	40	50	60	70	80	90	100	110	120		10	20	30
	feet																	
6	500	96	97	96	96	96	95	93	90	88	86	81	77	73	71	85	92	93
4	5,000	96	98	98	98	96	96	92	84	85	82	79			76	90	96	
5	10,000	92	93	93	92	89	84	81	77						72	84	93	
2	15,000	87	89	89	84	79	74	71							66	75	84	

The reduction in breath-holding time seen with decreased atmospheric pressure when the subjects breathe ambient air is remarkably similar to the percentage reduction in the calculated oxygen tension of the alveoli. This effect is not appreciably disturbed by preliminary spontaneous hyperventilation and the associated loss of carbon dioxide. This is in accord with the observations of Flack (4), Schneider (5) and Ferris (8, 13). With prolonged exposure to high altitude changes are seen in the breath-holding time as a result of changes in the acid-base balance (1).

It was of interest to note that the breath-holding time furnished a "bioassay" of the air-oxygen mixing regulators produced for the Army Air Forces designed to maintain the alveolar oxygen at that obtaining at ground level. However, while the regulator is calculated to deliver such a mixture up to 33,000 feet, the

breath-holding time was reduced at altitudes above 23,000 feet. This reduction could not be attributed to leakage around the oxygen mask, since the fit of the masks was carefully checked. This unexpected reduction of the breath-holding time between 23 and 33 thousand feet deserves further investigation.

The utility of the breath-holding time as a practical measure for use in escape at high altitude is obviously limited, as indicated in our centrifuge experiments. Such a maneuver would preserve the oxygen already present in the lungs during a free fall through the higher reaches of the atmosphere where serious anoxia might occur. However, it is apparent that the work attendant upon escaping from an airplane when dressed in heavy flying clothing, as well as the reduction brought on by the anxiety and excitement of the situation, would seriously limit the utility of the breath-holding maneuver. The ready availability of a supplementary oxygen supply is indicated.

It is apparent that the study of the breath-holding time furnishes a very useful technique for the study of the respiratory mechanism in normal man and under various conditions of physiological stress.

SUMMARY

1. Breath-holding time is increased by breathing oxygen, and after hyperventilation. It is decreased by reduced alveolar oxygen tension and by short bursts of exericse.

2. The usefulness of the breath-holding time in the study of practical and theoretical problems involving the function of the respiratory mechanism is discussed.

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THE EFFECT OF STIMULATION OF THE CAROTID SINUS REGION ON ABSORPTION OF CHLORIDE FROM THE SMALL INTESTINE

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It has been shown by Bernthal and Schwind (1) that there is a reflex vasoconstriction in the intestine in response to excitation of carotid and aortic chemoreceptors. These investigators used basically different procedures to elicit chemoreflex reactions. Chemical changes were brought about first by changing the composition of the inspired air, and second by the intravenous injection of chemical agents. In both instances a distinct constriction was produced in the intestinal vasculature. The vessels of the intestine behaved in the same manner as those of the spleen, submaxillary gland and the limb. These authors pointed out that this common response would be expected on the basis that carotid and aortic chemoreceptor stimulation initiates generalized excitation of thoracolumbar vasomotor efferents.

In view of the possible relation between vasoconstriction in the intestine and the rate of absorption it was considered worthwhile to investigate the effect of continuous carotid sinus stimulation on absorption from the small intestine of the mammal.

METHODS. The experiments were performed on barbitalized dogs (300 mgm. sodium barbital per kilo intravenously), which had been fasted 24 hours. One-hundred cubic centimeters of isotonic sodium chloride solution were placed in a Moreau loop of the lower portion of the small intestine and left for 30 minutes.

Thirteen dogs, used for the control experiments, had an average blood pressure of 122.9 mm. Hg. In order to rule out the factor of differences in blood pressure, these control animals were selected so that the blood pressure range was comparable to that of the experimental group.

Absorption was studied in 15 dogs while the carotid sinus region was continuously stimulated electrically for 30 minutes by placing an appropriate electrode directly into the carotid sinus. A Harvard inductorium was used which operated on a 7 volt current; the coil distance was from 3 to 6 cm. The average blood pressure of this group, while the carotid sinus was being stimulated, was 124 mm. Hg.

At the end of 30 minutes the sodium chloride solution was removed from the intestinal loop and the volume carefully measured. The amount of chloride in the solution was quantitatively determined by the Van Slyke modification of the Volhard method.

RESULTS. The data, table 1, show that there is a statistically significant reduction in the absorption of fluid and chloride in the experimental group. It is

of interest to note that the difference in the average blood pressure values between the control and experimental group was only 1.1 mm. Hg.

DISCUSSION. The results clearly indicate that less chloride and fluid were absorbed in the experimental group than in the control animals. Of several possible theories which might be offered to explain these findings, some of the more pertinent will be considered.

One factor which may be significant is the change in permeability of the intestinal epithelium following stimulation of the autonomic nerves. Gellhorn and Northup (2) in 1933, studying the frog's intestine perfused with isotonic glucose

TABLE 1

Effect of stimulation of carotid sinus region on absorption of chloride from intestine

CONTROL			EXPERIMENTAL		
Blood pressure	Fluid absorption	Chloride absorption	Blood pressure	Fluid absorption	Chloride absorption
mm. Hg	%	%	*mm. Hg*	%	%
103.4	23	49	111.4	17	32
104.6	51	61	112.8	73	78
116.0	70	75	115.0	37	44
117.4	52	66	117.8	16	40
120.2	57	62	118.2	52	57
121.2	56	64	119.4	50	59
121.4	82	—	119.6	55	60
129.2	43	49	121.4	43	49
129.8	62	68	122.0	54	64
131.7	68	74	122.2	36	55
133.0	56	63	124.4	57	64
134.0	51	58	127.8	45	58
135.2	82	88	135.2	17	35
			136.8	28	42
			156.4	50	54
Av...... 122.9	57.9	64.8	124	42	52.7

Av. differences: fluid, 15.9% ("p" 0.02); chloride, 12.02% ("p" 0.01); blood pressure, 1.1 mm. Hg.

solution, reported that absorption of glucose was altered by stimulation of the autonomic nerves. The blood vessels supplying the intestine were simultaneously perfused at a constant rate with Ringer's solution. They interpreted their results to mean that stimulation of autonomic nerves alters cellular permeability by means of humoral agents.

These authors had shown previously that acetylcholine and adrenaline had antagonistic effects on the transport rate across the intestinal epithelium. In high concentrations, adrenaline increased the rate of transport of glucose whereas acetylcholine decreased it; in lower concentrations the effects were reversed.

The conclusions of Gellhorn and Northup may not be applicable to our work, since they were studying absorption of glucose from the frog's intestine whereas

our work was concerned with chloride absorption from the mammalian intestine. It is a possibility, of course, that a humoral agent was responsible for the decreased amount of absorption of sodium chloride from the mammalian intestine; however, it does not seem a likely explanation.

The question of anoxia is to be considered, since there was a vasoconstriction of the intestinal vessels. It has been shown by Van Liere and Sleeth (3) that a moderate degree of anoxic anoxia has no effect on absorption of chloride from the intestine of the dog. Marked degrees of anoxia may actually increase chloride absorption (unpublished work). There is no reason to feel that there was a high degree of anoxia in the course of our experiments, and further if there had been, the absorption of chloride presumably would have been increased instead of decreased.

The most plausible explanation for the decreased absorption of chloride during stimulation of the carotid sinus region is the reflex constriction of the intestinal vasculature and the consequent decreased blood supply to the intestine. There may be contributing factors, but they are presumably less important than the decreased blood supply produced by reflex vasoconstriction. We do not know the actual degree of vasoconstriction which takes place. According to Bernthal and Schwind (1) the potential capacity for vasomotor response upon the part of the intestinal vessels and their nervous controlling mechanism is great. They stress, however, that the intensity of stimulation required to implement this capacity fully appears to be high and is brought into action only under extreme conditions.

In our experiments fairly strong faradic stimulation was used, and it is logical to suppose that considerable vasoconstriction was produced in the arteriole bed, and as a consequence, the blood flow through the capillary region was considerably reduced. The authors recognize, however, that there is not necessarily a direct relation between the amount of blood flow and the amount of absorption from the intestine.

Lastly, it should be emphasized that the control animals had been selected which had a blood pressure range within that of the experimental group. This was deemed necessary, because it has been shown in our laboratory (work to be published) that the level of the systemic blood pressure may significantly influence absorption from the small intestine.

SUMMARY

The absorption of isotonic sodium chloride solution from a Moreau loop of the lower portion of the small intestine was studied in 13 barbitalized dogs. The solution was left in the intestine 30 minutes. The average blood pressure of these dogs was 122.9 mm. Hg.

Absorption was studied for the same length of time in 15 dogs while the carotid sinus region was continuously stimulated electrically. The average blood pressure of this experimental group was 124 mm. Hg.

The percentage fluid and chloride absorbed by the control dogs was 57.9 and 64.8 respectively. In the experimental group the percentage absorption was

42 and 52.7 respectively. The latter group showed a statistically significant reduction of fluid and chloride absorbed.

The constriction of the intestinal vasculature and consequent decreased blood flow through the capillary region produced by stimulation of the carotid sinus region probably caused the lessened amount of absorption in the experimental group.

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THE SIZE AND FUNCTION OF THE HUMAN HEART AT REST IN SEMI-STARVATION AND IN SUBSEQUENT REHABILITATION[1]

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There is a widespread belief, maintained by most of the current textbooks in physiology, that the heart is resistant to undernutrition and does not undergo important degeneration or functional changes in starvation or prolonged underfeeding like the other tissues of the body. This idea has even been incorporated into a general principle proclaiming the wisdom of nature in protecting the most "vital" organs in starvation (cf., e.g., Foster, 1895; Evans, 1945). The current textbooks on cardiology generally make no mention of starvation effects, apparently having accepted the opinion of Vaquez (1924) in his authoritative textbook: "The conclusion is that inanition has no harmful effect on the heart" (p. 290).

The major purpose of the present paper is to present results of studies on the heart and circulation in 32 young men who, after several months of control observation on a normal diet, underwent controlled starvation to the extent of losing about one-fourth of their body weight in six months. Thereafter they were studied for three months during controlled rehabilitation and 12 of them for an additional two months on diets of their own choosing. Limited observations were made on 21 of these men after 32 to 35 weeks of rehabilitation and on a few of them about 20 weeks later. A brief general sketch of the experiment has been published (Keys, 1946). The electrocardiographic studies are reported separately (Simonson, Henschel and Keys, 1947).

In the subsequent presentation the control period is designated simply as "C", and the stages of semi-starvation are referred to as S5, S12, etc., the number referring to the number of weeks of semi-starvation. Similarly, the rehabilitation period is designated as R6, R12, etc., meaning the number of weeks following the end of semi-starvation.

Subjects and conditions. The subjects were volunteers from Civilian Public Service who were judged to be free from signs or history of significant physical

[1] The work described in this paper was initiated under sponsorship of the Brethren Service Committee, Elgin, Illinois, the Service Committee of the Society of Friends, Philadelphia, the Mennonites Central Committee, Akron, Pennsylvania, the John and Mary R. Markle Foundation, New York, the Sugar Research Foundation, New York, the National Dairy Council, operating on behalf of the American Dairy Association, Chicago, and the Home Missions' Board of the Unitarian Society, Boston. Later, this work was supported, in part, under the terms of a contract, recommended by the Committee on Medical Research, between the Regents of the University of Minnesota and the Office of Scientific Research and Development. During the later stages of rehabilitation this contract was transferred to the Office of the Surgeon General, U. S. Army.

or physiological abnormalities. They ranged from 20 to 33 years of age and were of sedentary to moderately athletic habits.

During a three-months' period of pre-starvation control and thereafter during semi-starvation and subsequent rehabilitation, these men lived and worked in the laboratory and maintained a set schedule of activity corresponding roughly to that of light industrial work. Aside from the usual upper respiratory infections, which were neither unusually frequent nor severe, there were no complicating illnesses.

The semi-starvation diet was patterned after the customary famine diets of Europe, i.e., the major food items were coarse cereals and potatoes, with cabbage and turnips supplying a fair amount of bulk. Dairy products, meats and fats were provided in only token amounts. No protein or vitamin supplements were given except in rehabilitation where the diet, for the first three months, was essentially unchanged except in quantity.

Besides the loss in body weight, these men developed the classical signs of starvation—weakness, fatigue, edema, depression, bradycardia, polyuria—and these rather slowly regressed in rehabilitation. There were no unequivocal signs of either protein or vitamin deficiency; even suggestive indications of vitamin deficiencies were few, slight and of dubious significance.

Methods. Cardiac measurements were made on roentgenkymograms. The subjects, in the resting state, briefly stood for the anterior-posterior view exposure. The cardiac silhouettes, in both ventricular systole and diastole, were drawn and both gross volumes and stroke were calculated following the method of Keys *et al.* (1940). Transverse diameter was measured in systole as was the long diameter; the latter was simply taken as the longest diameter in the anterior-posterior view. All measurements were corrected for triangular distortion. The major anatomical axis was drawn according to visual judgment and the angle of inclination of the heart was measured as the departure from the horizontal. An example of these tracings and measurements is given in figure 1.

Body weight was measured in the nude before breakfast. Basal metabolism was measured with a closed-circuit apparatus, using two periods of eight minutes each on every occasion. Venous blood pressure was measured with a saline manometer directly communicating by way of a 20-gauge needle with a vein in the antecubital fossa; the zero point was taken to be 10 cm. from the top of the litter on which the subject lay in the supine position.

Results. *Pulse rate and arterial pressure.* The most obvious changes relating to the heart and circulation were those in the pulse rate and blood pressure. Mean values in the basal state of rest are summarized in table 1. These subjects, as is commonly found with normal young men in this laboratory, were characterized by relatively slow heart rates and low blood pressures in the basal state. The averages at C were 55.19 beats per minute and 106.53/69.91 mm. Hg.

In the first few weeks of semi-starvation the blood pressure was unchanged but the heart rate became progressively slower, the most extreme bradycardia

being observed at S13 when the average heart rate was 35.31. Four men had rates of 28 on repeated manual counts of 1 minute each and one man had a rate of 24. Thereafter until the end of semi-starvation the heart rate very gradually rose, averaging 37.31 at S23. In the first 12 weeks of rehabilitation

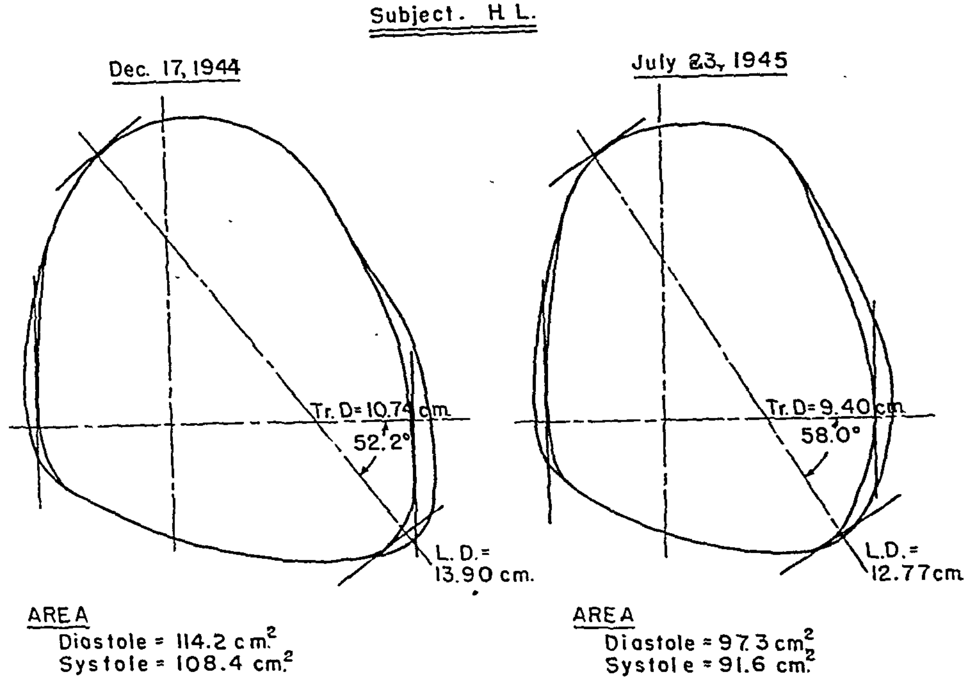

Fig. 1. Cardiac tracings and measurements made from roentgenkymograms of subject 1 during the control period (12/17/44) and at the end of semi-starvation (7/23/45). *Tr.D.* = transverse diameter, *L.D.* = long diameter. The diastolic and systolic areas are calculated from the planimeter tracings of the heart shadow outline.

TABLE 1

Mean values for 32 men for pulse rate and blood pressure (mm. Hg) in control (pre-starvation) and after 5, 13, 20 and 23 weeks of semi-starvation. All measurements made in basal rest

ITEM	CONTROL	S5	S13	S20	S23	R12
Body weight, kgm.	69.39	63.33	56.60	53.88	52.83	58.76
Pulse rate	55.19	40.78	35.31	36.75	37.31	49.66
Systolic B.P.	106.53	106.03	99.19	98.47	94.69	99.91
Diastolic B.P.	69.91	68.46	68.22	70.47	64.50	68.25
Pulse pressure	36.62	37.57	30.97	28.00	30.19	31.66

the heart rate slowly returned toward normal but still only averaged 49.66 at R12.

The response of the blood pressure to semi-starvation was less dramatic but definite signs of developing hypotension were seen by the middle of semi-starvation when the body weight had declined about 15 per cent on the average;

at this time the pulse pressure had also definitely decreased by about 15 per cent. As semi-starvation continued the systolic blood pressure continued to fall slowly with little or no further change in the pulse pressure. In the first 12 weeks of rehabilitation the recovery in both systolic blood pressure and pulse pressure was incomplete.

After 12 weeks of rehabilitation, when the men were allowed to eat as much as they wished, there was a rapid rise in the pulse rate to reach a level of relative tachycardia in 2 weeks and then falling slowly to be at about the prestarvation level at R20. Mean values for 12 men are given in table 2. Basal pulse rate measurements on 21 of the men at R34 gave an average of 58.71; this is significantly higher than the control mean of 54.90 for these same men.

In later rehabilitation (after R12) the blood pressure showed very little alteration. For 21 men the mean values at C and R34, respectively, were 106.6/69.9 and 107.4/71.1.

TABLE 2

Mean pulse rates and blood pressures in the basal state of the 12 men who were continuously observed through 20 weeks of rehabilitation

ITEM	CONTROL	S24	R6	R12	R14	R16	R20
Pulse rate	56.1	37.8	42.7	52.3	70.9	64.1	57.3
Systolic B.P.	105.3	92.7	97.2	101.5	106.8	104.9	104.0
Pulse pressure	35.1	29.4	30.7	32.3	34.3	34.9	35.4

Size, position and stroke. During the first 12 weeks of semi-starvation, the overall size of the heart for the whole group of 32 men diminished at almost the same rate as the reduction in body weight. While the body weight decreased by 17.4 per cent, the estimated heart volume decreased by 15.7 per cent. During the next 12 weeks, however, the rate of loss in heart size was considerably less so that the total period of 24 weeks produced an average loss of heart volume of 17.1 per cent while the corresponding loss in total weight was 24.0 per cent, with the ratio of heart size to body weight loss being 0.71.

The reduction in heart volume was accompanied by a sharp reduction in transverse diameter but by only a small decrease in overall length of the heart (long diameter). This may be explained, at least in part, by the changing position of the heart. The heart assumed a considerably more vertical position in the chest in starvation, the average difference in the angle of the major axis being 8.5° at S12 and 5.7° at S24. It should be observed that the transverse diameter represents a different proportion of the heart breadth when the axis angle is changed.

In rehabilitation, as shown by table 3, the cardiac dimensions were not back to the control level after 12 weeks (R12) but had slightly surpassed the control values at R20. The angle of the anatomical axis behaved in the same general fashion and the parallelism of all the cardiac dimensions with the changes in body weight was fairly close. There was a tendency for the return in heart

size to proceed more rapidly than the recovery in body weight, but the difference was of doubtful statistical significance.

The stroke volume of the heart decreased in starvation almost exactly in proportion to the total systolic volume of the heart. During rehabilitation, however, the recovery in stroke volume lagged considerably behind that in heart volume as shown in table 3. From the low point at S24 the increase at R20 was 26.6 per cent in total systolic volume, but the stroke volume increased only 9.3 per cent in these 20 weeks.

TABLE 3

Size of the heart in ventricular systole in rest, together with anatomical heart axis, body weight and basal pulse rate. The stroke volume is calculated from the difference between diastole and systole by the method of Keys et al. (1940). Means and standard deviations (S.D.) for 12 men in control, after 24 weeks of semi-starvation, and after 12 and 20 weeks of rehabilitation.

ITEM	C		S24		R12		R20	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Transverse diam., cm.	11.44	0.92	10.03	1.00	11.08	0.98	11.79	1.21
Transverse diam., %	100	8.0	87.7	10.0	96.8	8.8	103.0	10.2
Long diam., cm.	13.64	0.53	12.96	0.79	13.60	0.72	14.05	0.66
Long diam., %	100	3.9	95.0	6.1	99.7	5.3	103.0	4.7
Systolic volume, cc.	575.3	74.1	470.2	83.2	551.0	78.9	594.9	88.1
Systolic volume, %	100	12.9	81.7	17.7	95.6	14.3	103.4	14.8
Body weight, kgm.	67.7	5.15	51.7	3.34	58.2	3.77	70.8	4.44
Body weight, %	100	7.6	76.5	6.5	86.2	6.5	104.9	6.3
Pulse rate	56.1	6.09	37.8	5.15	52.3	6.61	57.3	5.92
Pulse rate, %	100	10.9	67.5	13.6	93.2	12.6	102.1	10.3
Stroke volume, cc.	66.9	5.44	54.8	8.83	57.4	10.12	59.1	9.26
Stroke volume, %	100	8.1	81.9	16.1	85.8	17.6	89.5	15.6
Axis, ° from horiz.	46.8	4.65	51.2	5.25	49.0	5.39	44.9	6.41

Physical work of the heart. Under the present resting conditions, a first estimate of the relative work done by the heart at the several periods of the experiment is provided by the simple product of the minute volume and the mean arterial blood pressure. Table 4 (line 7) shows that after six months of semi-starvation the physical work of the heart per minute in rest was reduced to half of the control value; in rehabilitation the cardiac work increased slowly, being only 90 per cent of the control level at R20.

The present discussion has omitted reference to the electrocardiographic findings. The duration of systole, measured either as the Q-T interval or as the time between the first and second heart sounds, was much prolonged by semi-starvation and remained long at R12. This means that the rate of the work of the heart during actual contraction of the ventricles was even more greatly affected than indicated by lines 6 and 7 in table 4. The mean mechanical systole duration was 0.322 seconds in the control period, 0.385 at S24 and 0.370 at R12 for the 12 men considered in table 4. Accordingly, the rate of

accomplishment of external work during systole at S24 can be calculated to be only 41 per cent as much as in the control period; the corresponding rate for R12 was 67.5 per cent of the control value (see table 4, line 8).

The figures for the work of the heart noted above do not include the kinetic work of imparting velocity. At the relatively slow blood velocities involved here, the kinetic work is only a very small fraction of the total physical work and so may be neglected in approximate calculations. The calculations in table 4 (line 9) indicate that the kinetic work of the heart per stroke declined at S24 to 57.3 per cent of the control value and was still only 64.9 per cent of C at R12. Calculating the rate at which kinetic work was done during the systolic contraction, the values at S24 and at R12 were, respectively, only 47.9 and 56.5 per cent of the control value (table 4, line 11). Finally, the kinetic work per minute at S24 and R12, respectively, was 38.6 and 60.5 per cent of C (line 10).

TABLE 4

Cardiac function in rest in the control period, after 24 weeks of semi-starvation, and after 12 and 20 weeks of rehabilitation. Mean values for the 12 men considered in table 2. In calculating the total cardiac work it is assumed that kinetic work comprises 3% of the total.

LINE NO.	ITEM	CONTROL VALUE	S24		R12		R20	
			Abs.	% C.	Abs.	% C.	Abs.	% C.
1	Cardiac output, L./min.	3.75	2.07	55.2	3.00	80.0	3.39	90.4
2	Mean art. press., mm. Hg	87.8	78.0	88.8	85.3	97.2	86.8	98.9
3	Mechanical systole, sec.	0.3222	0.385	119.6	0.370	114.9		
4	Diastolic heart vol., cc.	621.4	508.0	81.7	590.6	95.0	635.7	102.3
5	cc. stroke/100 cc. diast. vol.	10.7	10.8	100.9	9.6	89.7	9.3	86.9
6	Rel. pressure work, per stroke	100		72.7		83.3		87.2
7	Rel. pressure work, per minute	100		49.0		77.6		89.1
8	Rel. pressure work, rate in systole	100		41.0		67.5		
9	Rel. kinetic work, per stroke	100		57.3		64.9		
10	Rel. kinetic work, per minute	100		38.6		60.5		
11	Rel. kinetic work, rate in systole	100		47.9		56.5		
12	Rel. total work, per stroke	100		72.2		82.7		
13	Rel. total work, per minute	100		48.7		77.1		
14	Rel. total work, rate in systole	100		41.2		67.2		

With these values it is possible to estimate the relative total physical work of the heart, assuming that the kinetic work is something like 3 per cent of the total. The resulting values for total work are given in lines 12, 13 and 14 of table 4.

The functional state of the heart is sometimes estimated from the relation between stroke output and the diastolic size of the heart, on the general principle that the invocation of Starling's Law—increasing diastolic fiber length to ensure effective contraction—is an indication of developing failure. In table 4 (line 5) it is seen that the amount of blood ejected in systole per unit of diastolic volume was not decreased in starvation but that in rehabilitation

the diastolic heart size increased out of proportion to the stroke output. The result is the surprising indication that the heart was relatively closer to failure in early rehabilitation than in starvation. Actually, as we shall see, there were other indications that this was the fact.

Circulation versus metabolism. The most important consideration in evaluating the effective functions of the heart is the relation between the circulation and the metabolic requirements of the tissues. In starvation there is a very marked reduction in the oxygen demand. The details of the metabolism in this experiment will be reported elsewhere, but here we may note that, on the average, the total oxygen consumption per minute in rest was only 64 per cent as great at S24 as before starvation. The reduction in metabolic rate was, therefore, considerably more than the corresponding change in either stroke or total heart volume. It is necessary, however, to allow for the changes

TABLE 5

Circulation and metabolism in rest in the control period, after 24 weeks of semi-starvation, and after 12 and 20 weeks of rehabilitation. Column headings; U = upright, S = supine position. Mean values for the 12 men considered in tables 2 and 3. Bracketed values calculated as indicated in the text.

LINE NO.	ITEM	CONTROL		S24		R12		R20	
		U	S	U	S	U	S	U	S
1	Blood O_2 cap., cc./100 cc.		20.40		15.97		17.00		19.58
2	Arterial O_2, cc./100 cc.		19.44		15.17		16.15		18.60
3	Cardiac output, cc. O_2/min.	729	(801)	314	(346)	485	(538)	631	(694)
4	Metabolism, cc. O_2/min.	(274)	228	(174)	145	(223)	186	(287)	239
5	Ven. O_2 return, cc./min.	455	573	140	201	262	352	344	455
6	Ven. O_2, cc./100 cc. blood	12.13	13.91	6.76	8.81	8.73	10.66	10.14	12.20
7	Ditto, % of control	100	100	60	63	72	77	84	88
8	Ven. O_2 sat., %	59.3	68.0	42.3	54.9	51.3	62.7	51.8	62.3
9	Ditto, % of control	100	100	71	81	86	92	87	92
10	"Safety ratio," line 5/line 4	1.66	2.51	0.80	1.39	1.17	1.89	1.20	1.90
11	"Safety ratio," % of control	100	100	48	55	70	75	72	76

in pulse rate and in the oxygen capacity of the blood before finally deciding how well the heart maintained the circulation in relation to the metabolic need. The essential data and calculations are summarized in table 5.

The items of calculation in table 5 require some explanation. The metabolism was not measured at the moment of exposure of the roentgenkymogram but was measured separately in the supine position. The relation between heart volume and stroke volume remains the same in both positions according to Nylin (1933) but metabolism and total output of the heart are differently affected. In the upright (standing) position the metabolism is increased from 15 to 20 per cent compared with the supine position, but the cardiac (minute) output actually falls. In a series of 11 normal persons Grollman (1928) found the average changes in the upright position compared with the supine to be +17.8 and −5.3 per cent for metabolism and cardiac output, respectively. In this laboratory we have obtained rather similar results, using the foreign gas method for cardiac output, and these relations seem to be reasonably constant. Accordingly, therefore, it is proper to correct the cardiac output to

the supine position or to correct the metabolism to the upright position. We have made both calculations. For correcting metabolism from supine to upright we used the factor 1.2, i.e., 20 per cent, and for the correction of cardiac output from upright to supine we used the factor 1.1, or 10 per cent. These calculated values are indicated in brackets in lines 3 and 4 of table 5. The identity of procedures and calculations from period to period means that whatever may be the uncertainty as to absolute values, relative comparability should be assured.

In starvation the venous saturation declined about 25 per cent (upright 29, supine 19 per cent) but the oxygen content of the venous blood fell much more abruptly. If we consider the amount of oxygen returned per minute to the heart by the venous blood, the decline amounted to 69 per cent in the upright position and 65 per cent in the supine position. Perhaps the most useful comparison is in terms of the metabolic rate at the time. The venous (i.e., unused) oxygen returned to the heart per minute would cover the metabolic demand at the same moment about twice as long in the control period as after 24 weeks of semi-starvation (lines 10 and 11, table 5). At R12 this "margin of safety" was still only about three-quarters as great as before starvation and there was little further improvement at R20.

The venous pressure. In the measurements of venous pressure we were able to get suitable control data on 12 subjects who were also resident in the laboratory throughout the experiment, but were maintained in a normal state of nutrition. Two sets of measurements, separated by an interval of five months, were made on the control subjects. On the first occasion the average venous pressure for the 12 control men was 9.7 cm. of saline; the average for the second occasion was 10.3 cm. These values agree well with other "normal" measurements in the literature (e.g., Burch and Soderman, 1939); we have used the mean of these 24 control measurements—10.0 cm.—as the control reference.

The summarized data are given in table 6. At the end of semi-starvation the venous pressure was not more than half the normal value. It increased slowly in the first 12 weeks of rehabilitation and then rose sharply to surpass the control level at both R16 and R20. There was an indication that the high point was reached at R16 and thereafter the average tended to return toward the normal control level but the difference between R16 and R20 was not statistically significant.

The functional significance of an abnormally low venous pressure raises interesting but puzzling questions of both theoretical and practical types. For the present, however, it would seem safe to suggest, first, that a low venous pressure centainly does not imply cardiac incompetence, at least on the right side of the heart, and, secondly, that a rising venous pressure may be some indication of a relatively less perfect competence. From this viewpoint it could be suggested that the heart was functioning less well in rehabilitation, particularly at R16 and R20, than in semi-starvation.

The cause of the changes in venous pressure is not clear. In the first place,

it is not related to an inadequacy of total blood volume. As will be reported elsewhere, the total blood volume was relatively stable throughout the entire experiment, the averages for the several periods not varying outside the general range of 5500 to 6000 cc. There was, in starvation, a prolonged diastolic period so the time available for blood to enter the heart was increased. But at R12, when the venous pressure was still very low, the duration of diastole was actually somewhat less than in the control period. The significant points are given in lines 4, 5 and 6 of table 6.

TABLE 6

Venous pressure after 24 weeks of semi-starvation and after 6, 12, 16 and 20 weeks of rehabilitation. Mean values for 32 men and for 12 men as indicated. Control value in brackets is the mean for measurements on two occasions in 12 comparable normal men. Diastolic duration is the difference between total cardiac cycle duration and the duration of mechanical systole, the latter taken to be the time between the first and second heart sounds.

LINE NO.	NO. MEN	ITEM	CONTROL	S24	R6	R12	R16	R20
1	32	Ven. pressure, cm. saline	(10.0)	4.80	5.34	6.27		
2	12	Ven. pressure, cm. saline	(10.0)	4.75	5.48	6.46	11.42	11.00
3	12	Ditto, % of control	100	47.5	54.8	64.6	114	110
4	12	Diastolic duration, secs.	0.77	1.21		0.68		
5	12	Ditto, % of control	100	157		88		
6	12	Line 3 x line 5 x 10^{-2}	100	75		57		

TABLE 7

Cardiovascular function in later rehabilitation. Mean values for 21 men except for R20 where the values are given for 12 men. The "oxygen pulse" (line 6) gives the cubic centimeters of oxygen metabolized per heart beat. The "circulatory index" (line 7) attempts to allow for the changing oxygen capacity of the blood as indicated in the text.

LINE NO.	ITEM	C	S24	R12	R20	R34
1	Body weight, kgm.	69.36	52.53	58.08	70.84	75.79
2	Basal pulse rate	54.90	37.48	49.95	58.70	58.71
3	Systolic blood pressure, mm. Hg	106.6	94.5	100.3	104.4	107.4
4	Diastolic blood pressure, mm. Hg	69.7	64.3	68.8	69.1	71.1
5	Pulse pressure difference, mm. Hg	36.9	30.2	31.6	35.3	36.3
6	Oxygen pulse	4.20	3.84	3.64	4.11	4.09
7	Circulatory index	209.3	242.6	214.9	210.3	199.3

Later rehabilitation. After 20 weeks of rehabilitation the subjects dispersed from the laboratory and were under no dietary or activity regulation. About three months later (R34) it was possible to reassemble 21 of the men at various medical centers where follow-up examinations were made. Early in the morning, before breakfast, basal pulse rate, blood pressure and metabolism were measured, a blood sample was taken, and an electrocardiogram was recorded; this was the same general schedule used in the earlier parts of the experiment. These data are summarized in table 7.

The basal pulse rate, which at R20 slightly but definitely surpassed the control value, was unchanged at R34; in other words, in later rehabilitation the pre-starvation (control) bradycardia was less marked or it could be said there was a mean relative tachycardia of about four beats a minute. Systolic and diastolic pressures were above the control values but only to an insignificant degree.

It was believed that comparison between the metabolism and the pulse rate might be useful. Such comparison is sometimes made in the form of an "oxygen pulse" calculation in which the (oxygen) metabolic rate is simply divided by the pulse rate, thereby yielding the amount of oxygen provided per heart beat. This oxygen pulse fell sharply from the control value in starvation, was still lower at R12 and seemed to be returning to normal rapidly at R20. At R34, however, the oxygen pulse had not continued to improve and was, actually, a trifle lower than at R20. For the present purpose, at least, it might seem better to make allowance for differences in the hemoglobin concentration of the blood, on the general theory that this is another factor in the oxygen balance. Obviously we may write the complete equation:

I. Metab. Rate = Stroke Vol. $\times$ Pulse Rate $\times$ O^2 Cap. $\times$ (Art. Sat.—Ven. Sat.) then:

$$\text{II.} \qquad \frac{\text{Metab. Rate}}{\text{Pulse Rate} \times O_2 \text{ Cap.}} = \text{Stroke Vol.} \times \text{(Art. Sat.–Ven. Sat.)}$$

Accordingly, we have calculated the values for the left side of this question for use as a "circulatory index", the average values for which are given in line 7 of table 7. This index rose sharply in semi-starvation and tended to make a fairly rapid and complete return in the first 20 weeks of rehabilitation. At R34, however, the index had continued downward and was below the level at C. This difference between R32 and C was of questionable statistical significance, being just short of the 5 per cent level.

Diet in rehabilitation and cardiovascular function. During the first 12 weeks of rehabilitation the 32 men were divided into matched groups which were maintained on different levels of calories, proteins and vitamins. Four groups of eight men each were maintained on caloric levels differing by 400, 800 and 1200 calories per man-day from the group on the lowest rehabilitation intake; the latter averaged 2464 Cal. Each of these caloric groups was subdivided into matched sub-groups of four men each, one sub-group receiving daily vitamin pills while the other sub-group received blank pills identical in appearance. Finally, each of these sub-groups in turn was subdivided into two sections of two men each, the men in one section receiving a bread enriched with soy bean and milk proteins, while the other men received bread of equal caloric and vitamin content but not fortified with extra protein.

This factorial design of this part of the experiment allows the comparison of four caloric levels, all other factors being constant, for groups of eight men each. Further, we may compare two groups of 16 men each whose regimens

were identical save for the fact that one group received only the vitamins in their food while the other group had this same diet plus supplementary vitamins corresponding to the "minimal requirements" of the U. S. Food and Drug Administration. The "Hexavitamin" tablets used provided each man the following daily supplements: Vitamin A—2500 International Units, thiamine—1.0 mgm., riboflavin—1.5 mgm., niacin amide—10.0 mgm., ascorbic acid—37.5 mgm., and vitamin D—200 International Units. Finally, we may compare two groups of 16 men each whose diets were calorically the same and whose vitamin intakes were identical, but whose daily protein intakes differed by 20.7 grams per man.

TABLE 8

Caloric intake and recovery in cardiovascular items in the first 12 weeks of rehabilitation. Means and standard deviations (S.D.) for 8 men in each group. In each case the "% Recovery" is calculated from the change from S24 after 12 weeks of rehabilitation (Δ R12) as compared with the change from the control period (C) to S24.

ITEM	CALORIC GROUP	C	S24	ΔS24		ΔR12		% Recovery
		M	M	M	S.D.	M	S.D.	M
Basal heart rate, beats per minute	+1200 Cal.	58.0	38.0	−20.0	8.8	+13.4	6.4	67.0
	+800 Cal.	53.8	36.9	−16.9	6.2	+11.4	7.3	67.5
	+400 Cal.	56.4	38.0	−18.4	4.4	+13.1	3.4	71.2
	Basal diet	52.6	36.4	−16.2	5.0	+9.8	3.8	60.5
Basal arterial pulse pressure, mm. of Hg	+1200 Cal.	34.6	30.2	−4.4	6.7	+3.1	7.6	70.5
	+800 Cal.	36.5	28.9	−7.6	6.7	+3.2	6.2	42.1
	+400 Cal.	38.2	30.8	−7.4	4.0	+0.9	3.1	12.2
	Basal Diet	37.1	30.9	−6.2	8.7	−1.4	7.1	−22.6
Basal venous blood pressure, cm. of saline	+1200 Cal.	(10.0)	4.81	(−5.19)		+2.12	2.14	(40.9)
	+800 Cal.	(10.0)	4.98	(−5.02)		+1.00	1.85	(19.9)
	+400 Cal.	(10.0)	4.44	(−5.56)		+1.81	1.86	(32.5)
	Basal Diet	(10.0)	4.99	(−5.01)		+0.94	2.15	(18.7)

The effects of the differences in caloric intakes in rehabilitation are the most clear and interesting. As we should expect, the body weight gains systematically reflect the caloric differences, but the several items of cardiovascular function show variable responses. Several items are summarized in table 8. The basal heart rate increased to much the same extent regardless of the caloric level in rehabilitation. The blood hemoglobin concentration was similarly relatively independent of the caloric level in rehabilitation. In contrast, the systolic arterial pressure and the basal pulse pressure changed in close relation to the caloric level of the diet. Finally, the "recovery" in venous blood pressure was greatest in the highest caloric group and least in the group on the basal diet, but the intermediate sequence was irregular.

No clear and consistent effects from the vitamin or protein supplementation appear on detailed analysis. Some of the items are summarized in table 9.

More significant results might be expected from such supplements at low or high caloric intakes in rehabilitation; e.g., vitamins might be useful, or vice versa, at particular caloric intakes. If such relations exist, they are not readily demonstrated with the small numbers available for comparison in the subgroups here.

Peripheral circulation. In the literature on starvation there are constant references to the coldness of the skin and the subjective complaint of coldness and cold intolerance. These phenomena were prominent in the Minnesota Experiment but it might be questioned whether these are to be referred to circulatory or metabolic deficiency. Slight but unmistakable cyanosis of the nail beds was seen in all men in rest at one time or another in the semi-starvation period. In the period S16 to S24 cyanosis was constant in 27 of the 32 men. The cyanosis was not increased in exercise and was not severe at any time; it disappeared in the first few weeks of rehabilitation. There were some

TABLE 9

Effects of vitamin and of protein supplementation on recovery in some cardiovascular items. Mean values for the percentage of the changes in semi-starvation restored in the first 12 weeks of rehabilitation. U = no protein supplement, Y = with protein supplement. H = with vitamin supplement, P = no vitamin supplement.

ITEM	8 MAN GROUPS				16 MAN GROUPS			
	UH	UP	YH	YP	U	Y	H	P
Basal pulse rate	79.0	59.2	74.0	66.5	69.1	70.3	76.5	62.9
Basal systolic pressure	32.9	63.7	19.1	46.3	48.3	32.7	26.0	55.0
Basal pulse pressure	27.4	52.4	12.2	−26.2	39.9	7.0	19.8	13.1
Basal venous pressure	20.1	31.3	29.8	32.1	25.7	31.0	25.0	31.7

complaints that the extremities would "go to sleep" frequently but this was neither a marked nor a consistent finding. The general appearance was of a moderate deficiency in the peripheral circulation.

Other indications of the circulatory state. The edema which developed in 28 out of 32 of these men cannot well be ascribed to cardiac failure in view of the low venous pressure and the absence of other indications (cf. Keys *et al.*, 1946). There were no complaints of palpitation, tachycardia, dyspnea or pre-cordial distress in the starvation period or in early rehabilitation. The circulatory findings in work cannot be discussed here but, in general, they did not indicate any special cardiac or circulatory defect out of proportion to the capacity of the voluntary muscles.

Complaints of dizziness and "blackout" on rising suddenly were common after the first few weeks of semi-starvation and the men soon learned to be cautious on this score. Fainting is very frequently seen in starving people in natural famine (Zimmer *et al.*, 1944). Slower adjustments to altered posture were well maintained, however, as judged by tilt-table tests.

We have discussed the bradycardia of starvation elsewhere (Simonson, Hen-

schel and Keys, 1947). Without recourse to teleological argument, this bradycardia can be considered a valuable and protective adaptation. It is significant that the rate at which cardiac contraction work was done at S24 is 41 per cent of the control, but that the cardiac output was only reduced to 55 per cent. Marked bradycardia has been noted in practically all reports on semi-starvation but the present values are unusually low. The somewhat higher rates in the literature undoubtedly reflect the fact that less perfect relaxation was attained.

Discussion. *The heart size.* English and American textbooks of physiology, insofar as they make any reference to the effects of starvation on the heart, consistently state that the heart tissues are very little affected (e.g., Bard, p. 789, 1941; Best and Taylor, p. 1022, 1943; Evans, p. 765, 1945; Fulton, p. 1112, 1946; Parsons, p. 96, 1939; Roaf, p. 586, 1936; Wright, p. 764, 1940; Zoethout and Tuttle, p. 404, 1943). A bar diagram showing the percentage weight losses of the several organs is frequently employed. Apparently this bar diagram was first employed by Waller (1896, p. 256) to depict the data from a single starved cat reported by C. Voit (1866), in which the heart weight was only 2.6 per cent smaller than that of a single "control" animal. C. Voit (1894) later obtained quite a different finding in starved dogs.

The idea that the heart is safeguarded against starvation effects can be traced to Foster (1895) who "interpreted" Voit's earlier data. There has been confusion between *C.* Voit and *E.* Voit. The latter reported, in 1905, some results indicating a 16 per cent loss in heart weight in a starvation experiment; Starling referred to E. Voit in his first edition (1912) but in more recent editions C. Voit (1866) has been substituted. Bard (1941) cites E. Voit instead of C. Voit. C. Voit himself placed no emphasis whatever on the singularity of the heart weight in his one cat; moreover he cited and apparently accepted the work of Chossat (1843) who carried out many starvation experiments and found an average loss of 44.8 per cent of the heart weight while the voluntary muscles lost 42.3 per cent.

A very long series of careful studies all agree that the heart undergoes extensive atrophy and degeneration in starved rats, rabbits, dogs, guinea pigs, chickens, crows, pigeons, man *and* cats. As for the latter, Sedlmair (1899) found losses of 44 to 55 per cent in the heart weight in starvation. References to many other concurring papers are given by Morgulis (1923) and Jackson (1925).

In starved human beings autopsy data provide convincing proof that starvation results in a reduction in cardiac tissue which at least approaches the proportions of the general loss of body weight. The detailed report on 459 consecutive autopsies of famine victims in India (Porter, 1889) shows a reduction in heart size of the same order of magnitude as the loss in total body weight. The analysis of heart weights in 1534 autopsies by Bean and Baker (1919) also shows a very marked reduction in cardiac tissue more or less in proportion to the degree of emaciation of the rest of the body. In 571 autopsies on children the reduction in heart size was considered to be nearly in proportion to that of the whole body (Bovaird and Nicoll, 1906). Krieger's (1920) findings in 123

autopsies of emaciated patients indicate estimated weight losses for the heart of 18 to 45 per cent when the body weight losses amounted to 35 to 48 per cent; on the average the ratio of percentage of heart weight loss to percentage of body weight loss was 30:41 or about 0.75.

In the Minnesota Experiment we only have estimations of the volume of the intact heart but it is clear that this overall volume diminished by something like 20 per cent. This does not necessarily mean an equivalent change in the bulk of the heart tissue, since the heart volume includes the blood remaining in the chambers at ventricular systole. The first suggestion would be that, since the heart in starvation is obviously weakened, the systolic ejection might be less complete than normal. This would mean a larger residue of blood and hence an underestimate of the shrink of the actual cardiac tissue. The opposing argument that systolic ejection is unusually complete and effective would be difficult to entertain.

Obviously, discussion of these points cannot settle the issue precisely with the evidence now at hand. We can only observe that the indicated heart volume changes here are roughly in agreement with the changes in heart weight versus body weight observed by others at autopsy (e.g., Krieger, 1920). Furthermore, while the present data may have underestimated the shrink of cardiac tissues, it is not conceivable that the change may have been *overestimated* to any significant degree.

Starvation versus rehabilitation. We have remarked on some indications that the cardiovascular function was perhaps less effective or had a smaller reserve in the rehabilitation period than in the starvation period. During semi-starvation it was observed that the men frequently stopped physical effort with complaints of weakness or fatigue but seldom complained of being out of breath or otherwise suggested respiratory-circulatory distress. In rehabilitation, however, dyspnea was a common complaint, particularly in the period R12 to R20 when the most rapid weight gains were made. In this latter period relative tachycardia was very frequently observed both in rest and in work. The venous pressure rose, on the average, to a slight degree above our normal average but more significant was the fact that 4 out of 13 men examined at R14 to R16 had venous pressures of 15 cm. of water or more.

At the time of most rapid weight gain there was also a dramatic rise in the basal metabolic rate. It would be reasonable to suggest that the heart, which had been able to support the much lower metabolic load in late starvation and early rehabilitation, tended to demonstrate its weakness when the metabolic demand was increased. One subject had a short spell of what appeared to be actual heart failure which would seem to prove the point. This case is instructive.

Subject 130 during semi-starvation had exhibited edema and his cardiovascular and other responses had been no different than the other men. During the first 12 weeks of rehabilitation his edema abated but tended to recur occasionally like many of the other men. After R12 this subject immediately gorged himself and in spite of warnings continued to eat at the general level of 7,000 to 10,000 Cal. daily. At the same time he increased his physical activi-

ties somewhat. In two weeks he had a sudden, massive return of edema and complained of dyspnea. At this time it was found he had moderate edema of the face, extremities and the sacral region, blood pressure of 116/80, pulse rate 80, hemoglobin 14.2 grams per 100 cc., white count of 6,350, and a total plasma protein concentration of 6.3 grams per 100 cc. with a ratio of albumin to globulin (A/G) of 1.75. The venous pressure had risen by 10 cm. of saline solution, the heart had sharply increased in size and the basal metabolism had increased more than 30 per cent in two weeks. Treatment consisted of bed rest, fluids limited to 1500 cc. daily, diet about 3000 Cal., and ammonium chloride. After a day or two diuresis began and he quickly lost ten pounds. All signs of heart failure were gone and he was discharged in a week. This subject had no further difficulty.

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SUMMARY

1. Measurements of the heart and its function were made on 32 normal young men in rest before, during, and after six months of semi-starvation in which one-fourth of the body weight was lost. These men developed the typical signs associated with severe famine.

2. During semi-starvation the heart decreased in all dimensions and assumed a more upright position in the chest. The total volume reduction averaged 16 per cent and the stroke volume reduction 18 per cent. The systolic blood pressure fell 12 mm. and the diastolic pressure 6 mm. The venous blood pressure was not more than 50 per cent of the normal level. The heart rate averaged 37 beats per minute. There was slight cyanosis of the nail beds but no dyspnea.

3. During semi-starvation the physical work done by the heart, compared with the control period, averaged 72 per cent per beat, 49 per cent per minute and 41 per cent per unit of time in contraction. The mean oxygen saturation of the venous blood fell from 20 to 30 per cent and the oxygen content from 35 to 40 per cent. The margin of safety represented by the ratio of the metabolism to the oxygen circulated in the blood fell to half the normal average.

4. In the first 12 weeks of rehabilitation on controlled diets the heart size returned almost to the control level but all other characteristics showed only relatively small recovery, being generally somewhat less than in proportion to the recovery of lost body weight.

5. During these first 12 weeks of rehabilitation neither vitamin nor protein

supplementation had significant effects on the recovery of cardiovascular function.

6. After 20 weeks of rehabilitation, the last eight being on unlimited diets, the body weight was fully restored and the total heart size was slightly greater than before starvation. But at this time the work done by the heart per minute was still about 10 per cent below the control and only about half of the lost margin of safety had been restored.

7. After 32 weeks of rehabilitation, the last 20 weeks being on unlimited diets, the majority of cardiovascular measurements were back at the control level but the oxygen pulse was not completely at the former value.

8. During the first few months of rehabilitation the stroke output per unit of diastolic heart volume decreased compared with both the control and the semi-starvation values. There were some other signs that the heart was closer to failure in early rehabilitation than in starvation. One case of congestive failure in rehabilitation responded promptly to conservative management.

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PLASMA VOLUME AND THIOCYANATE SPACE IN FAMINE EDEMA AND RECOVERY[1]

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Edema, commonly known as famine, hunger or war edema, is an outstanding characteristic of persons who have undergone prolonged caloric undernutrition. The great loss of cellular materials, the disappearance of adipose tissue and the reduction of muscle tonus in starved persons make it difficult to estimate the magnitude of the fluid changes clinically.

This paper is a report on the results of measurements of plasma volume by the dye method (T1824) and of the thiocyanate space, as an estimate of extracellular fluid, in men who were experimentally brought to a severe state of undernutrition and then rehabilitated. Thirty-two normal young men subsisted on a European type of famine diet for 24 weeks during which time they lost an average of about one-fourth of their body weight. Thereafter they were maintained on restricted rehabilitation diets for 12 weeks and then allowed to eat at will. Observations in the rehabilitation phase of the experiment were continued for a year after the end of the semi-starvation; at the time of the final observations these men were fully restored to their pre-starvation state with regard to all of the many functions and characteristics tested.

After about 8 weeks on the famine regimen a few of the subjects showed signs of clinical edema and as the semi-starvation continued the frequency and severity of the edema cases increased. By 24 weeks 28 out of the 32 men had exhibited edema, although in some instances the edema appeared to fluctuate. Grading the edema on a total scale of 0 to 4+, the majority of the men were graded 1+ (minimal pitting edema of the shins), 7 to 10 (depending on the observer and the occasion) were graded 2+, and 2 men were graded 3+; none of the men was graded 4+ (general anasarca with ascites) at any time. Besides the edema, these men exhibited the typical signs of severe starvation as seen in European famine and in the German concentration camps—weakness, anemia, depression, polyuria, bradycardia, lowered metabolism and cold intolerance. Further

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details are provided in separate papers dealing with other aspects of the Minnesota Experiment (Keys *et al.*, 1947; Brozek *et al.*, 1947; Simonson *et al.*,1947).

METHODS. The plasma volume was estimated at regular intervals by the dye dilution (T1824) method described by Gibson and Evans (1937) and Gibson and Evelyn (1938). The estimates were made in the basal state in the morning and were in all cases preceded by a minimum of two hours of inactivity and one-half hour of supine rest. During most of the testing periods, the estimates were done in duplicate four to seven days apart so as to gauge the reliability of the results. All blood samples—the dye-free, and the 20, 30, and 40 minute post-injection samples—were drawn in oiled syringes and 20 gauge needles with a minimum of stasis from the antecubital veins, and the blood was immediately delivered into paraffined centrifuge tubes containing approximately 2 mgm. of heparin. Hematocrit values were determined on the dye-free and the 20-minute dyed samples. The theoretical L values were determined by plotting the corrected L values against time and extrapolating back to zero (dye injection) time.

In the tests at 24 weeks of semi-starvation and at all the rehabilitation periods, thiocyanate space was estimated simultaneously with the plasma volume by using an injection mixture of the blue dye (T1824) and sodium thiocyanate. The injection mixture contained per 10 cc. (the volume injected) approximately 10 mgm. of T1824 and 500 mgm. of sodium thiocyanate. The absolute concentration of T1824 and sodium thiocyanate was accurately determined for each batch of injection mixtures. Blood samples for the thiocyanate space determinations were taken before and at 10, 20, 30, 40, and 50 minutes after the injection of the T1824-thiocyanate mixture. The basal and the 20, 30, and 40 minute post-injection blood samples were the same ones that were used for the plasma volume estimations. The determination of the concentration of the sodium thiocyanate in the plasma samples and the calculation of the thiocyanate space were made according to the procedure of Bowler (1944). This method was modified so that the intensity of the color in the final solution was determined by means of the Evelyn photoelectric colorimeter with a 440 mμ filter.

All the post-injection plasma samples were corrected for the amount of thiocyanate-like material that was present in the basal sample. The concentration of thiocyanate in the five post-injection samples was plotted against time. If a distinct break occurred in the disappearance curve, that point was taken as the equilibrium concentration of thiocyanate in the body fluids and was used in the calculation of the thiocyanate space. In the cases where no distinct break occurred in the disappearance curve the mean of the equilibrium times for the other subjects in the same group was arbitrarily used. The thiocyanate space was calculated as liters of extracellular fluid and as per cent of the body weight. No correction was made for the thiocyanate that may have entered the red blood cells.

RESULTS. *Plasma volume.* The principal results for the plasma volumes in the control and semi-starvation periods, and through the eleventh week of

rehabilitation, are summarized in table 1. The total plasma volume in liters at any one time will be referred to as the absolute plasma volume whereas the volume in cubic centimeters per kilogram of body weight will be called the relative plasma volume.

Two independent trials during the control (pre-starvation) period gave absolute plasma volume averages of 3.130 and 3.165 liters, or relative plasma volumes of 45.01 and 45.57 cc. per kgm. The relative plasma volume continued to rise during semi-starvation until after 24 weeks of semi-starvation (S24), when the body weight had decreased about 24 per cent, the relative plasma volume was 41.7 per cent greater than in the normal (control) state; whereas the absolute plasma volume at this time had increased only 8.9 per cent. The total blood volume at S24 had decreased by 8.6 per cent, reflecting the 21.0 per cent decrease in the hematocrit value and the considerable degree of anemia that had developed. There was, actually, very little overlapping of the ranges of relative plasma volumes at the control period and S24; the ranges were 35.3 to 55.9 and 49.9 to 81.9 cc. per kgm., respectively.

TABLE 1

Total and relative plasma volumes in liters and cubic centimeters per kilogram of body weight, respectively, and hematocrits taken on two occasions in the control period, at 12 (S12) and 24 (S24) weeks of semistarvation and 5 (R5) and 11 (R11) weeks of rehabilitation. Mean values for the numbers of men indicated.

PERIOD	CONTROL 1	CONTROL 2	S12	S24	R5	R11
Number of men	32	30	18	32	14	16
Body weight kgm.	69.54	69.45	58.90	53.45	54.49	57.95
Plasma vol. liters	3.130	3.165	3.176	3.410	3.232	3.090
Plasma vol.cc./kgm.	45.01	45.57	53.92	63.80	59.31	53.32
Hematocrit %	46.29	46.68	39.85	36.60	38.16	39.51

The first few weeks of restricted rehabilitation were accompanied by a slight decrease in the relative hydremia. At 11 weeks of rehabilitation (R11) the body weight was still 16.7 per cent below the control weight, but the absolute plasma volume was back to the pre-starvation value. At R11 the mean relative plasma volume was 18.4 per cent above the previous control value, that is, about the same as at S12 when the body weight was also very similar.

Later rehabilitation data were obtained on only a few of the subjects. A clear idea of the relative hydremia in the later stages of rehabilitation can, however, be obtained from four subjects on whom more complete data are available for 19, 35, and 55 weeks of rehabilitation. The data are summarized in table 2. The decrease of the relative plasma volume from the twelfth to the nineteenth week of rehabilitation was rapid. Although the averages of the four men for both the absolute and relative plasma volumes were within normal limits from R19 through R55, some of the subjects in this group showed marked deviations from the averages.

Thiocyanate space. Measurements of the thiocyanate space were made at

the end of semi-starvation (S24) and at various times during rehabilitation. For an estimate of the pre-starvation values, control measurements were made on 21 normal young men who resided with the starvation subjects, but who were in a normal state of nutrition. The data for the semi-starvation and control groups are summarized in table 3.

TABLE 2

Total and relative plasma volumes in liters and cubic centimeters per kilogram of body weight, respectively, for 4 subjects at control, 24 weeks of semi-starvation (S24) and 11 (R11), 19 (R19), 33 (R33) and 55 (R55) weeks of rehabilitation.

SUBJECT NO.	CONTROL		S24		R11		R19		R35		R55	
	liters	cc./kgm.	liters	cc./kgm.	liters	cc./kgm.	liters	cc./kgm.	liters	cc./kgm.	liters	cc./kgm.
2	3.430	46.5	4.250	74.2	4.420	72.3	3.183	42.4	3.536	45.3	3.612	50.4
109	3.102	40.0	3.938	66.8	3.442	53.4	3.343	43.0	3.314	42.3	3.037	37.8
112	2.784	44.9	2.835	54.1	2.907	49.8	2.976	46.1	2.786	41.5	2.929	44.3
130	3.540	50.2	4.349	81.9	3.472	62.6	3.229	43.3	(3.100)*	(41.2)*	2.908	80.9
Mean...	3.214	45.4	3.843	69.3	3.560	59.5	3.182	43.7	3.184	42.6	3.121	43.4

* Calculated by the missing data formula.

TABLE 3

The thiocyanate space at 24 weeks of semi-starvation (S24), 5 (R5), 11 (R11), 19 (R19) and 35–55 (R35–55) weeks of rehabilitation. The control data are for a comparable group of 21 young men who were on an adequate diet.

ITEM	CONTROL	S24	R11	R19	R35–55
Mean SCN space % body wt.	23.50	33.98	30.53	23.75	23.51
Max. SCN space % body wt.	26.3	38.7	34.3	25.8	26.1
Min. SCN space % body wt.	20.2	30.3	25.9	22.3	21.3
S.D. ± SCN space % body wt.	1.65	2.84	2.37	1.29	1.43
Total liters of SCN space	17.13	17.88	17.93	16.88	17.06
Number of cases	21	17	16	7	13

The average thiocyanate space in the control group was 17.13 liters or 23.50 per cent of the body weight, with a rather narrow range of 20.2 to 26.3 per cent and a S. D. = ±1.65. Near the end of the semi-starvation period the thiocyanate space averaged 33.98 per cent of the body weight, range 30.3 to 38.7, S. D. = ± 2.84. There was, however, only a relative increase in the body hydration; actually the total absolute thiocyanate space remained remarkably constant throughout the entire experiment (table 3). After 11 weeks of restricted refeeding the thiocyanate space, as per cent of the body weight, decreased to 30.53 per cent; a decrease of about 10 per cent from the S24 high. With the institution of the unrestricted diet after R12 the thiocyanate space rapidly returned to the normal level.

The abnormality of the relative hydration in semi-starvation is best indicated by calculations of the thiocyanante space which is in excess of the normal proportion; this was taken to be 23.5 per cent of the body weight. If F is the fraction of the body designated by the thiocyanate space, and W is the body weight, then the excess hydration (E) in semi-starvation would be estimated as:

$$(1)\ E = FW - 0.235W$$

This calculation, however, neglects the fact that the mineral mass of the skeleton, which makes up about 4 per cent of the body weight, is substantially unchanged in semi-starvation. A more accurate formulation would then be: (2) $E = FW - 0.235\ (W - 0.04W_0)$, where W_0 is the body weight before starvation.

Equation (2) applied to the average data for S24, R11 and R19 yielded the results summarized in table 4. At the end of semi-starvation the subjects had in their bodies an average of 6.25 liters (13.8 lbs.) of excess extracellular fluid. The major portion of the excess hydration persisted through the first 11 weeks of restricted rehabilitation but had almost entirely disappeared at R19.

TABLE 4

Average excess hydration in liters and per cent of body weight at the end of semi-starvation (S24), 11 (R11) and 19 (R19) weeks of rehabilitation. These estimates refer solely to extracellular fluid.

ITEM	S24	R11	R19
Excess SCN space, liters.......	6.25	4.72	0.17
Excess SCN space, % wt.......	10.48	7.03	0.25

Thiocyanate space and clinical edema. The relation between thiocyanate space and the total mass of the body, or the mass of the body less thiocyanate space, is a measure of the extracellular hydration. In a normal state of health and nutrition the percentage of the total body weight represented by the thiocyanate space in young men was found to be relatively constant in 21 cases; as we have noted, the mean was 23.50, S. D. = ± 1.65; in 13 determinations in the starvation subjects after complete recovery, the mean was 23.51 per cent. The high degree of constancy of these results suggests that it should be feasible, in these young men, to consider the amount of thiocyanate space in excess of 23.5 per cent of the body weight as relative edema; in the present subjects the calculations indicate that at the end of semi-starvation (S24), 10.48 per cent of the body weight was "thiocyanate edema fluid."

In 18 subjects clinical estimates of edema were made by several observers at about the same time as the thiocyanate space was measured in them. The relation between the clinical estimates and the "thiocyanate edema fluid" is given in table 5. On the average the difference between the subjects who were clinically edema-free and those who were graded edema 2+ and 3+ corresponded to the difference between 7.8 per cent and 12.2 per cent of the body weight as excess thiocyanate space or thiocyanate edema fluid. This corresponds to an

average of about 2.3 kgm. (5.1 lbs.) of fluid as the difference between very marked edema and no recognizable edema. Equally interesting is the fact that in these men clinical edema could not be recognized until about 10 per cent of the body weight was made up of excess thiocyanate space.

Clinical character of the edema. We have already remarked on the general severity of the edema produced in this experiment but some further details are of interest. The edema was of the soft, dependent type as is generally the case with the edema seen in famine areas. In most of the men it was limited to the face and lower legs; in some cases one or both eyes would be almost swollen shut in the morning when they woke up; later in the day the feet and ankles would swell as the facial puffiness diminished. Repeated examinations failed to reveal any signs of either ascites or hydrothorax at any time; this again is similar to "natural" famine edema where accumulations of fluid in the body cavities is not common except in the presence of other complications.

TABLE 5

Relation between clinical edema and the percentage of the body weight represented by "thiocyanate edema fluid," i.e., thiocyanate space in excess of 23.5 per cent of the weight. The mean values tabulated are for the thiocyanate edema fluid as a percentage of the body weight. "No." is the number of men in the group.

EDEMA-FREE			EDEMA 1+			EDEMA 2+ TO 3+		
No.	Mean	Range	No.	Mean	Range	No.	Mean	Range
6	7.8	6.7–8.0	7	10.9	7.6–15.2	5	12.2	8.2–14.6

Some of the subjects developed accumulations of fluid in the knee joints; in several cases this was enough to be troublesome in walking. Systematic examinations toward the end of the semi-starvation period revealed a "floating" patella in 16 of the men (50 per cent). Excluding the 4 men who showed no clinical edema at any time, the incidence of definite edema of the knee joint was 57 per cent. In view of the rather moderate degree of general edema in these men this seems to be an unusually high percentage.

In rehabilitation clinical edema tended to disappear rather rapidly and, except for puffy eyes in the morning and occasional brief episodes, was gone after 6 weeks. When unlimited feeding was resumed after 12 weeks several men had brief recurrences of edema; unfortunately the body fluids were not estimated in these individuals during these episodes.

Intravascular versus extravascular hydration. When calculated per unit of body weight, the mean plasma volume and thiocyanate space rose in semi-starvation to very much the same extent—41.7 and 44.6 per cent, respectively. The question then arises as to the correlation between the relative hydration of blood and the tissues. Since the thiocyanate space includes the plasma, it is necessary to subtract the plasma volume from the extravascular fluid space (thiocyanate space) if the two distinct body compartments are to be compared.

The correlations for the individual data summarized in tables 1 and 3 have

been made. For 34 pairs of observations made on subjects in the normal nutritional state, the coefficient of correlation between the plasma volume and the extravascular fluid volume, both expressed in cubic centimeters per kilogram of body weight, was +0.421. The coefficient of correlation was +0.215 for the 17 pairs of observations at S24. For the entire data of 85 pairs of observations, including controls, semi-starvation and rehabilitation, the coefficient of correlation was +0.706; the correlation surface is given in figure 1.

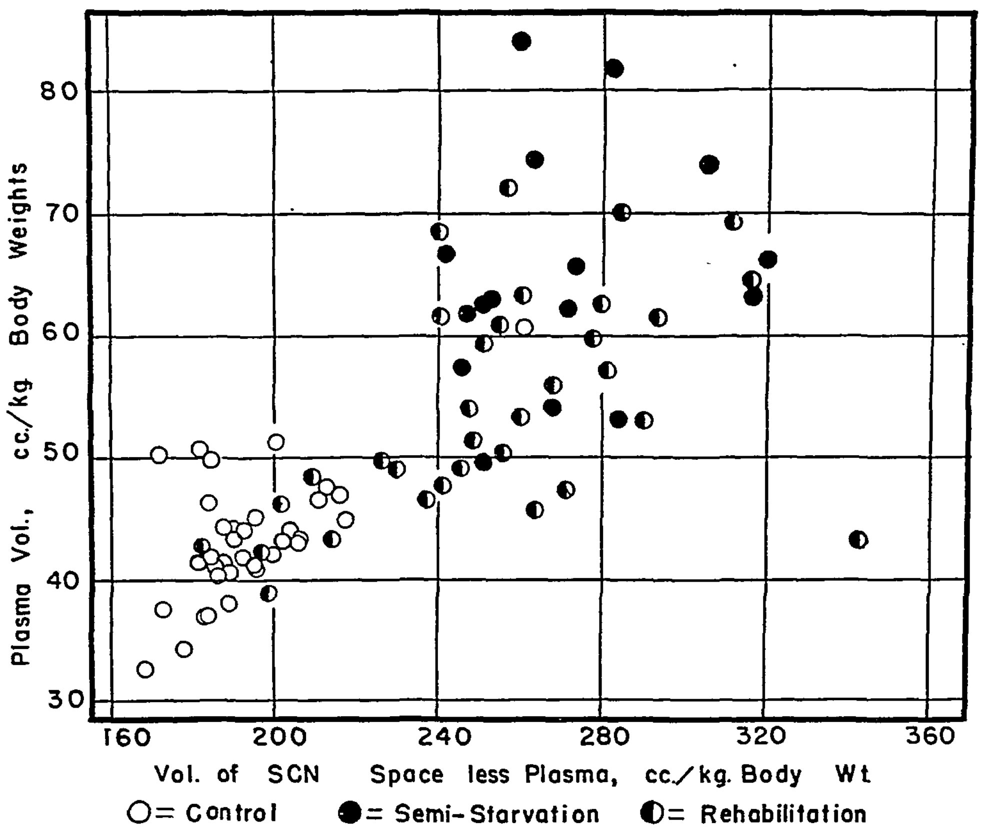

Fig. 1. The correlation between plasma volume and extracellular fluid volume, both expressed in cubic centimeters per kilogram of body weight, for 85 pairs of observations including control, semi-starvation and rehabilitation values.

Discussion. Analyses of the bodies of starved animals uniformly show a relative increase in hydration but there have been few serious attempts to examine the phenomenon further. The fact itself was amply documented 40 years ago (cf. Morgulis, 1923). Experimental demonstrations of the production of edema by protein deficiency and the finding of hypoproteinemia in cases of famine edema in the Orient led to the general belief that famine edema is simply a manifestation of a low colloid osmotic pressure in the plasma. It now appears that, though this mechanism may play a rôle, the edema of simple starvation is not due solely to this change (Keys *et al.*, 1946).

Certainly famine edema can appear without any marked hypoproteinemia

or even hypo-albuminemia and the severity of the edema does not bear any clear relation to the concentration of proteins in the plasma (e.g., Jansen, 1920; Youmans *et al.*, 1932; Kostyal, 1935; Jimenez Diaz *et al.*, 1942; Gounelle *et al.*, 1942; Warembourg *et al.*, 1942; Nicaud *et al.*, 1942; Morgan *et al.*, 1946). In the Minnesota Experiment the plasma protein concentration in starvation fell, on the average, only 0.73 gram per 100 cc. and the ratio of albumin to globulin was even less affected. Relative congestive heart failure was ruled out by the fact that the venous pressure did not rise but actually fell to the low average of 4.8 cm. of saline. The absence of any loss of capillary impermeability to colloids was shown by the fact that the edema fluid, obtained by direct drainage, contained extremely little protein—less than 0.2 gram per 100 cc.

Whenever people subsist in a grossly negative caloric balance and undergo a rapid and large loss of body tissue, edema appears. A second basic fact is strongly indicated by the present results. The edema tends to be the expression of only a relatively excessive hydration. The absolute thiocyanate space remains remarkably constant and the "blame" for the edema might equally well be placed on the wasting away of the cellular elements of the body. Both the plasma and the extravascular fluid tend to maintain their absolute volumes at the pre-starvation levels.

The question might be asked: What mechanism is there which would operate to cause the fluid mass of the body to shrink in proportion to the cellular elements? In other words, why should we expect that there would not be a rise in the relative proportion of fluid? We may conceive of the normal (pre-starvation) relation between fluid and tissue in the body to have been established by growth and development in a normal architecture of fixed structures and elastic forces. The diminution of the cells does not necessarily reduce the space available for fluid; indeed, elastic hindrances to expansion of that space should be reduced.

There are few data in the literature on starvation which bear directly on the present results. In 5 moderately underweight persons Perera (1946) reported a mean plasma volume which was 17.5 per cent above the normal average when calculated per square meter of body surface but when the data were expressed in cubic centimeters per kilogram of body weight the excess amounted to only 5 per cent. More useful data were obtained by Mollison (1946) on 6 males and 9 females studied at the Belsen prison camp at the time of liberation (table 6). Edema classified as 1+ to 3+ was present in 4 of the males and 5 of the females. The plasma volume averaged 64.1 cc. per kgm. of body weight at the time of examination. This may be compared with our average of 63.80 cc. per kgm. of body weight for 32 men after 23 weeks of semi-starvation. It will be noted that our average before starvation was 45.01 cc. per kgm. and this is exactly the same as Gregersen's (1944) normal average.

Mollison's data are incomplete regarding weight loss; 4 of his patients were included in a group of 11 Belsen inmates for whom an average weight loss of 38.8 per cent was estimated on the basis of statements from the patients. The actual weight loss was probably somewhat smaller than this, since starved persons

invariably over-estimate their pre-starvation weights and their starvation losses. In any case it may be presumed that the average weight loss for the Belsen inmates involved in the plasma volume work was something between 30 and 38 per cent. It is interesting to attempt the calculation of weight loss for the Belsen males from the plasma volume data. Assuming the absolute plasma volume was substantially constant and had amounted to 45 cc. per kgm. of body weight before starvation, the pre-starvation weight of the men would be estimated to have averaged 64.5 kgm. This indicates a loss of 22 kgm. or 34.1 per cent of the original body weight.

There are no data in the literature on the thiocyanate space in famine victims and we have been unable to discover any efforts in this direction in extensive correspondence and personal contacts with workers in the field.

TABLE 6

Body weights in kilograms, plasma volume in liters and plasma volume in cubic centimeters per kilogram of body weight of the males and females for the total group, and for those with and without edema. The values were calculated from the data presented by Mollison (1946) for severely starved internees at the Belsen prison camp.

	TOTAL GROUP			WITH EDEMA			WITHOUT EDEMA		
	Body Wt.	Pl. Vol.	Pl. Vol.	Body Wt.	Pl. Vol.	Pl. Vol.	Body Wt.	Pl. Vol.	Pl. Vol.
	kgm.	*liters*	*cc./kgm.*	*kgm.*	*liters*	*cc./kgm.*	*kgm.*	*liters*	*cc./kgm.*
Males.....	42.5	2.90	68.4	42.5	2.83	66.4	42.5	3.05	72.5
Females...	38.4	2.22	59.7	42.1	2.24	55.3	33.8	2.20	65.2
Mean...	40.5	2.56	64.1	42.3	2.54	60.9	38.2	2.64	68.9

On the average, both plasma volume and thiocyanate space tend to remain absolutely constant in starvation. This does not mean, however, that these fluid compartments do not vary from time to time in starved individuals. In the Minnesota Experiment body weight was obtained under highly standardized conditions every morning on arising. Both short-time and long-period fluctuations occurred which can only be interpreted as sudden changes in the fluid balance. An example is shown in figure 2. These shifts were apparently spontaneous and could not be traced to alterations in activity or other changes; the rigid constancy of the regimen in the Minnesota Experiment left small room for accidental changes. Similar and greater individual fluctuations in weight, presumably involving changes in the extracellular fluid, are frequently seen in famine victims and, for that matter, in all kinds of edema.

The physical meaning of the thiocyanate space is still open to doubt. Although it is agreed that it is related to the extracellular fluid volume of the body, the question as to exact identity may be argued. Lavietes, Bourdillon and Klinghoffer (1936) found thiocyanate, sucrose and inorganic sulphate are all distributed through approximately the same fraction of body fluid and all show changes in the direction predicted when measures are used to change the extracellular space. In 13 good experiments on 5 normal men they got a range of 20.1 to 26.1 per cent of the body weight for the thiocyanate space; this is almost identical with the results here in a normal state of nutrition.

Winkler, Elkington and Eisenman (1943) studied 3 dogs with injections of thiocyanate, Cl^{38} and Na^{24} and found these to be distributed through approximately 36, 25 and 28 per cent of the body weight, respectively. They concluded: "The volume of distribution of sulfocyanate is therefore not a satisfactory absolute measure of the volume of the extracellular fluid. Under most conditions, however, it may be a useful relative measure, i.e., changes in its volume of distribution may reflect changes in extracellular fluid." But the distribution volume for thiocyanate they obtained is very much higher than found in normal man and also sharply distinct from that found in dogs by others. Mellors

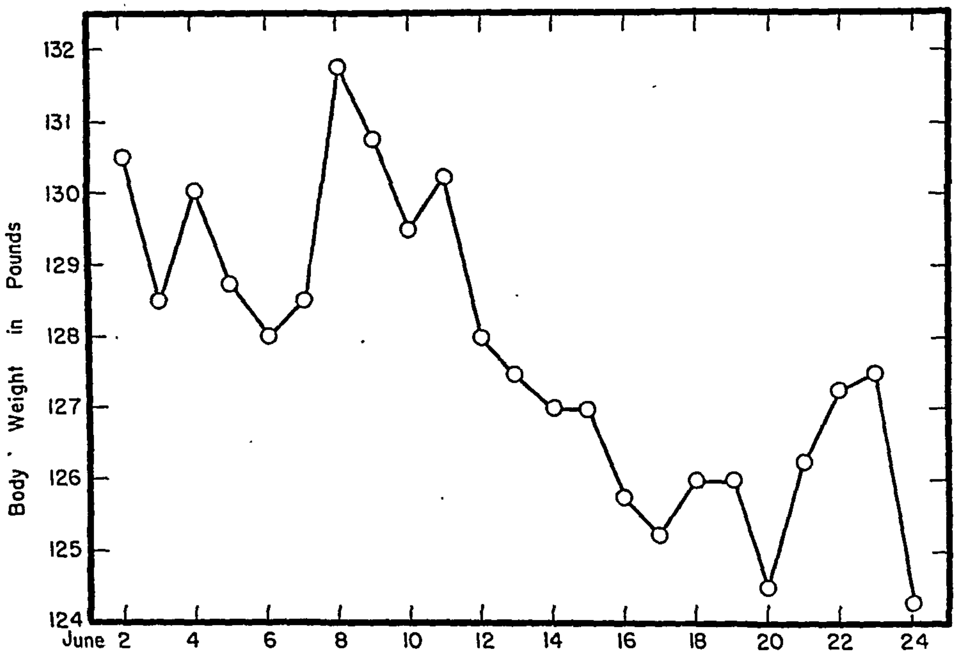

Fig. 2. Daily body weights for subject 1 during the fifth month of semi-starvation illustrating spontaneous daily weight variations.

et al. (1942) found average thiocyanate spaces in 2 series of 9 dogs each of 25.9 and 28.7 per cent of the body weight.

SUMMARY

1. Plasma volume (T-1824) and thiocyanate space were estimated in a group of young men during a control period, after 6 months of semi-starvation in which about one-fourth of the body weight was lost, and at intervals up to one year during rehabilitation. Clinical edema developed in 28 out of 32 subjects and the clinical picture was typical of European famine.

2. In control observations in the normal state of nutrition the plasma volume, in cubic centimeters per kilogram of body weight, averaged 45.01, S. D. = ±4.00. In the same condition the thiocyanate space averaged, in per cent of the body weight, 23.50, S. D. = ±1.65.

3. The absolute amount of plasma increased slightly during semi-starvation but promptly returned to normal when refeeding was instituted. The absolute thiocyanate space remained substantially constant at all times.

4. Relative plasma volume, in cubic centimeters per kilogram of body weight, increased by an average of 41.7 per cent during semi-starvation and was back to normal after 5 months of refeeding.

5. Thiocyanate space, in cubic centimeters per kilogram of body weight, increased by an average of 44.6 per cent in semi-starvation and was substantially back to normal after 5 months of refeeding.

6. In 85 pairs of observations covering the entire experiment the coefficient of correlation between plasma volume and extravascular fluid volume, both expressed as cubic centimeters per kilogram of weight, was +0.706.

7. Calculation of the thiocyanate space in excess of the normal proportion to body weight indicated that this "thiocyanate edema" was related to clinical edema but that clinical edema could not be discerned until the thiocyanate edema amounted to more than 8 to 10 per cent of the body weight.

8. The edema in simple caloric starvation is largely a reflection of a reduction in cellular mass without large change in the absolute amount of extracellular fluid.

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THE EFFECT OF INTRA-ARTERIAL INJECTION OF ADRENALIN UPON BLOOD FLOW OF THE HUMAN FOREARM[1]

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The rôle of the sympathetic nervous system and of adrenalin in the regulation of muscle circulation in man is not clear. That the problem is of considerable physiologic and clinical interest is evident from the recent widespread use of sympathectomy in peripheral vascular disease.

A great deal of information has been obtained from animal experimentation. According to McDowall "... the sympathetic constricts especially the vessels of the skin and intestines, while it dilates, usually, but not always, the vessels of the muscles and the heart" (1). Dilatation of blood vessels of the muscle by injection of small doses of adrenalin in animals has been reported repeatedly. Clark (2) and Roome (3) found a twofold effect—dilatation and constriction—of a single intra-arterial dose of adrenalin upon the blood flow in the skeletal muscle of anesthetized animals. Rein et al. (4) observed a constrictor action of adrenalin and of sympathetic impulses, in the anesthetized dog, if the muscle was at rest; in the contracting or hyperemic muscle, adrenalin injection and sympathetic stimulation respectively were ineffective or had a dilator effect.

In man, Grant and Pearson (5) concluded on the basis of intravenous injections of small doses that adrenalin was a true vasodilator for the muscle. Eichna and Wilkins (6) found that mild sympathetic stimuli produced little or no change in the circulation of resting human muscle. Stronger stimuli (which usually elevated the blood pressure) resulted in no change, in decrease, or most frequently in increase of blood flow of resting or hyperemic muscle. Barcroft et al. (7) showed that blocking the nerve supply to the human forearm with procaine effected an increased blood flow to the muscles. In a sympathectomized person, however, the nerve block had no influence upon the circulation. They concluded that muscle vessels, in the human, possess sympathetic constrictor fibres and tone. In a recent publication, Allen, Barcroft et al. (8)[3] report the results of intravenous, and in two cases, of intra-arterial infusion of adrenalin. The infusion of adrenalin into the femoral artery resulted in vasodilatation in the calf muscles.

The available studies of sympathetic and adrenalin effects upon the circulation in muscle are obviously confusing. Experiments on anesthetized animals have been criticized as being unreliable for vascular studies. Intravenous injections

[1] Part of the expense of this work was defrayed by a grant from the Martha Hall Foundation.

[2] Fellow of the Baruch Committee on Physical Medicine.

[3] This paper published in December, 1946, came to our attention after our experimental work was nearly completed.

and reflex sympathetic stimulation result in a mixture of systemic cardiovascular adjustments which make an analysis of direct local effects hazardous. It appeared to us that the effect of single intra-arterial injections of adrenalin in man offered the best approach to this problem.

METHOD. The plethysmograph of Abramson (11) was used for estimating blood flow. Determinations were made on the upper extremities of subjects with clinically normal peripheral circulation. Their age ranged from 23 to 54 years. They were seated, comfortable and relaxed, with the extremity proximal to the plethysmograph supported by a soft pillow. In all experiments, a basal period of complete rest for half an hour preceded the determinations. The room temperature during experiments was fairly constant at 22°C. and varied less than two degrees. The water temperature in the plethysmograph was maintained between 32°C. and 34°C.; in a few instances, it was kept between 35°C. and 37°C.

The recording apparatus consisted of a tambour with an ink writer. Timing was by means of a stop-watch. For the forearm measurements, the hand circulation was excluded by placing a tourniquet 4 cm. wide about the distal portion of the forearm close to its emergence from the apparatus. This remained in place for 25 to 30 minutes. Lewis has shown that this does not affect significantly measurement of forearm blood flow.

In all experiments control blood flow measurements were made. It was felt that variations of 20 per cent in successive blood flow determinations could not be regarded as significant of change in blood flow. Because of the type of recording device used, any marked drop in blood flow was represented in our records as a horizontal line which in the tables is designated "O" blood flow. This is not meant to indicate an absence of blood flow.

Intra-arterial injections were made into the brachial artery, distal to the collecting cuff and close (usually less than 1 inch) to the enclosed portion of the forearm. Very sharp, long bevelled, 22 gauge needles were used. Contrary to most reports the actual penetration of the arterial wall caused little if any pain or discomfort. If pain was produced in finding the artery, it was slight and transient.

When two substances were injected in succession, one arterial puncture was made, the needle then being left in place, and only the syringes interchanged. Substances injected were made up in normal saline within several minutes of use; the largest volume injected was 0.3 cc.

The procedures carried out were as follows:

1. Intra-arterial injection of adrenalin in the resting forearm; saline controls.
2. Intra-arterial injection of adrenalin during reactive hyperemia.
3. Intra-arterial injection of adrenalin after intra-arterial injection of histamine.
4. Simultaneous intra-arterial injection of adrenalin and histamine.
5. Adrenalin intravenously, measuring—a, forearm blood flow;—b, hand blood flow.
6. Reflex vasoconstriction, measuring—a, forearm blood flow;—b, hand blood flow.

RESULTS. 1. *Intra-arterial injection of adrenalin in the resting forearm.* In preliminary experiments, intra-arterial doses of 5 gamma adrenalin produced tremor, pallor and anxiety as well as rise in blood pressure. Doses of 2 gamma or less did not produce such effects although in those experiments in which it was measured the blood pressure showed a slight to moderate rise. In no instance where it was measured did the blood pressure fall after any dose of adrenalin.

Intra-arterial injection of 1–2 gamma adrenalin in six experiments on six subjects produced marked and immediate reduction in blood flow which was maintained for approximately ninety seconds and then returned gradually to normal. In most instances records were made for more than three minutes; the table gives the significant figures only. The flow curves during marked constriction show strikingly the disappearance of pulsations. In two instances,

TABLE 1

Intra-arterial injection of adrenalin

SUBJECT	RESTING BLOOD FLOW*	GAMMA ADRENALIN	BLOOD FLOW AFTER ADRENALIN†
W. S.	2.8	2.0	34 seconds:0 119 seconds:0
A. W.	5.4	2.0	47 seconds:0 125 seconds:3.3
A. M.	2.3	2.0	15 seconds:0 86 seconds:0.96
A. A.	2.3	1.5	48 seconds:0.7 176 seconds:2.4
K. H.	1.7	1.0	40 seconds:0 91 seconds:1.6
J. B.	1.6	0.5	12 seconds:1.8 70 seconds:1.4 188 seconds:1.5
S. W.	2.6	0.5	16 seconds:2.1 52 seconds:1.8 88 seconds:2.5
A. M.	2.0	0.1	15 seconds:2.0 45 seconds:1.5 75 seconds:1.8
K. H.	3.3	0.05	15 seconds:7.4 45 seconds:9.1 135 seconds:2.0
L. G.	1.3	0.05	45 seconds:4.0 74 seconds:1.6

TABLE 1—*Concluded*

SUBJECT	RESTING BLOOD FLOW*	GAMMA ADRENALIN	BLOOD FLOW AFTER ADRENALIN†
M. C.	2.7	0.03	15 seconds:3.5 75 seconds:4.0 135 seconds:3.2
B. S.	1.0	0.01	30 seconds:5.5 60 seconds:0.2 150 seconds:0.5
L. G.	2.5	0.002	12 seconds:6.9 92 seconds:2.4
L. G.	2.0	0.0002	13 seconds:6.8 34 seconds:4.8 107 seconds:0.7
J. N.	2.4	0.0002	15 seconds:5.4 40 seconds:3.7 179 seconds:1.9
L. G.	2.5	0.00002	15 seconds:2.7 30 seconds:3.2

* Blood flow in cc./min./100 grams tissue in all tables.

† Recorded at stated time after injection.

In all experiments observations at rest were made until three constant readings were obtained. Changes in blood flow were followed at ½ minute intervals until the flow returned to resting level. Fifteen to 20 readings were needed in each instance.

recording was made without the collecting cuff in order to demonstrate the marked reduction in the volume of the forearm.

With doses of 0.5 and 0.1 gamma in four experiments on three subjects there was no significant alteration in the blood flow. The variations tabulated are within the limits of error of the method.

Doses of 0.05 and 0.03 gamma increased the blood flow markedly in two instances and significantly in a third; the vasodilatation was transient. In one subject it was followed by a drop below the control level after 105 seconds.

Doses of 0.01, 0.002 and 0.0002 gamma adrenalin in five experiments on three subjects also produced vasodilatation. Its intensity was not different from that of larger dilating doses. It was followed in two experiments by a definite and in one other by a slight vasoconstriction.

A dose of 0.00002 gamma produced no significant change in the blood flow.

The average increase over resting blood flow by these doses of adrenalin was 198 per cent, varying from a minimum of 48 per cent to a maximum of 450 per cent.

It does apparently not reach the much higher levels seen by us during reactive hyperemia and known to occur during exercise.

Several facts brought out by these experiments deserve emphasis. The two

levels where adrenalin is apparently or actually ineffective are at 0.5–0.1 gamma and at 0.00002 gamma respectively. The vasodilator effect of adrenalin is maintained over a very wide range of low to extremely low concentrations. The constrictor action is only seen after large doses, and then within a narrow range. Their significance will be discussed.

In one subject, the effect of intra-arterial injection (brachial artery) of 0.01 gamma of adrenalin on the hand circulation was measured. A constrictor effect was observed.

The intra-arterial injection of 0.2 cc. saline produced no alteration in the forearm blood flow in seven subjects.

TABLE 2

Intra-arterial injection of adrenalin during reactive hyperemia

SUBJECT	TIME OF INJECTION AFTER RELEASE OF CIRCULATION	GAMMA ADRENALIN	BLOOD FLOW: REACTIVE HYPEREMIA	BLOOD FLOW AFTER ADRENALIN
S. W.	88 seconds	1.0	10.6	14 seconds: 0 83 seconds: 1.3 187 seconds: 1.7
S. W.	78 seconds	2.0	13.7	16 seconds: 0 111 seconds: 0.84 150 seconds: 2.0
J. B.	55 seconds	2.0	13.6	31 seconds: 0 78 seconds: 1.5 245 seconds: 2.6
S. M.	39 seconds	2.0	14.8	15 seconds: 13.0 68 seconds: 5.6 95 seconds: 1.8
S. M.	60 seconds	2.0	11.5	20 seconds: 11.8 75 seconds: 4.6 160 seconds: 3.5

2. *Intra-arterial injection of adrenalin during reactive hyperemia.* To determine how constrictor doses of adrenalin would act in presence of locally produced vasodilatation, a tourniquet was applied just distal to the collecting cuff, to occlude the arterial flow, and left in place for 10 minutes. At the end of this period it was released and a blood flow curve immediately recorded. Adrenalin 1–2 gamma was injected quickly. Table 2 shows the results of five experiments in which the adrenalin was introduced into the artery within 90 seconds. Preliminary experiments had shown that the peak flow after release was reached in approximately that period.

It is to be noted that when adrenalin was injected close to the peak flow (90 sec.) the constricting effect was immediate and clear cut. In two experiments in which injection was made within a minute after release of the tourni-

quet the blood flow dropped only gradually and not below usual resting levels. In a third experiment in which injection was made within one minute, a temporary vasoconstriction was followed by a slight dilatation.

These findings are difficult to interpret because of the number of factors involved, e.g., dosage, time of injection, effect of rate of blood flow on washing out and destruction of dilator substances or adrenalin.

The results suggest, however, that adrenalin produces full vasoconstriction when reactive hyperemia begins to subside. While hyperemia is reaching its peak the constrictor effect of adrenalin is not apparent.

3. *Intra-arterial adrenalin following intra-arterial histamine.* In preliminary experiments 4 gamma of histamine intra-arterially produced a well marked hyperemia which reached its peak in about 50 seconds and then fell off very sharply to approximately resting level.

TABLE 3

Intra-arterial injection of 1.0 gamma adrenalin after intra-arterial injection of 4 gamma histamine

SUBJECT	TIME OF INJECTION OF ADRENALIN*	RESTING BLOOD FLOW	BLOOD FLOW AFTER HISTAMINE†	BLOOD FLOW AFTER ADRENALIN
S. W.	49 seconds	2.8	14 seconds: 9.0	14 seconds:2.0 47 seconds:1.1 78 seconds:2.5
K. H.	35 seconds	2.7	12 seconds:13.7	8 seconds:2.7 45 seconds:1.3 79 seconds:0.8

* After histamine injection.

† Recorded at stated time after injection.

Because of the equivocal results noted in the previous experiments we used histamine to produce local vasodilatation. It is assumed that histamine or a histamine-like substance is one of the factors responsible for exercise hyperemia of muscle (9). Exercise was not used because clenching the hand with the plethysmograph attached to the forearm was attended by technical difficulties.

Table 3 shows that a constrictor effect was produced by adrenalin. The injections were made close to the time of peak flow produced by the histamine and the first blood flow determinations were recorded at a time when hyperemia is expected to be subsiding spontaneously.

4. *Intra-arterial injection of a mixture of adrenalin and histamine.* The reasons for adopting this step are obvious from the foregoing.

On three different occasions, in a single subject, increasing doses of adrenalin were mixed with a constant dose of histamine and the mixture injected intra-arterially.

The results are unequivocal; adrenalin even in dosage four times larger than necessary to produce a constrictor effect in resting muscle cannot overcome the dilator effect of the minimal effective dose of histamine.

The constrictor effect of the adrenalin, however, becomes manifest with the larger doses (2 and 4 gamma) after the histamine hyperemia has passed, indicating that the adrenalin was not altered by mixing with the histamine.

An interesting point in connection with these experiments is that in this subject the 4 gamma dose of adrenalin (mixed with histamine) produced no systemic effects. According to previous experience such a dose of adrenalin alone would produce pallor and tachycardia.

5. *Intravenous adrenalin measuring forearm blood flow and hand blood flow.* In six experiments our results confirmed the observations of previous observers (Grant and Pearson (5); Eichna and Wilkins (6); Barcroft et al. (7)) that intravenous administration of adrenalin in doses of the order of 1 to 2 gamma produced transient vasodilatation in the forearm circulation and constriction in the hand circulation without significant alteration in the blood pressure.

TABLE 4

Simultaneous intra-arterial injection of adrenalin and histamine

SUBJECT	GAMMA ADRENALIN	GAMMA HISTAMINE	RESTING BLOOD FLOW	BLOOD FLOW AFTER INJECTION
K. H.	1.0	4.0	2.5	5 seconds:8.8 18 seconds:5.0 108 seconds:2.6
K. H.	2.0	4.0	3.1	5 seconds:6.2 80 seconds:0.6 110 seconds:2.0
K. H.	4.0	4.0	3.6	5 seconds:7.5 20 seconds:0 50 seconds:1.3 110 seconds:3.2

6. *Reflex cold on forearm blood flow and hand blood flow.* Immersing the free hand momentarily in ice water produced uniformly in three subjects an immediate diminution in blood flow in the contra-lateral hand—a well known phenomenon.

In the same subjects the same procedure produced no significant change in the forearm blood flow.

In two subjects we attempted to determine the effect on the forearm circulation of prolonged (twenty minutes) exposure of the legs to water of 50°F. The results were equivocal.

Discussion. The forearm blood flow in man represents muscle circulation because of the preponderance of muscle to skin (4–5 to 1). It is safe to say that the direction of change of blood flow in the forearm is determined by what happens to the muscle circulation.

Our results leave no doubt that depending on the dosage, adrenalin may produce either constriction or dilatation in the circulation of the skeletal muscles in man.

Constriction is caused only by relatively large doses of adrenalin. The range of the constrictor action of intra-arterially injected adrenalin is very narrow. Doses of about 5 gamma give rise to systemic cardiovascular reactions, 1–2 gamma have an apparently pure local constrictor effect and 0.5 gamma is seemingly ineffective. Moreover, in presence of an effective amount of histamine and probably also during development of reactive hyperemia and exercise hyperemia, constrictor doses of adrenalin become ineffective.

Adrenalin produces vasodilatation in human muscle over a wide range of doses from low to extremely minute concentrations. The dilator action of adrenalin is not intense and is followed in some experiments by a constrictor effect.

Since no reliable data exist concerning the amount of adrenalin in the circulating blood, it is impossible to determine from our experiments whether a dilator (or a constrictor) effect should occur in muscle during a given situation of stress. This is of course also true for the effect of sympathetic impulses upon the blood flow of muscle in a given case.

It appears to us, however, that dilatation must be the chief action of adrenalin and of sympathetic impulses upon the blood vessels of the muscle.

The constricting and dilating effects of adrenalin on muscle blood flow can be considered an excitatory and inhibiting action on sympathetic effectors. This is, in our opinion, an erroneous interpretation. The sequence of constrictor action, apparent ineffectiveness, dilator action and final ineffectiveness of diminishing doses of adrenalin can be understood only on the basis of independent constrictor and dilator mechanisms. Both are stimulated simultaneously by adrenalin. With large doses of adrenalin the constrictor action predominates; with smaller doses it balances the dilator action. At low and very low concentrations the dilator action prevails. Large differences in responsiveness of the constrictor and dilator mechanism to adrenalin must be assumed. The irregular appearance of constriction after dilatation in our experiments (and the observation of dilatation in the wake of constriction by other investigators) may be the result of a longer maintained effect in one of the two activated mechanisms. Dilator action is known to occur with all doses if the constrictor mechanism is paralyzed by ergotamine. Whether the dilator mechanism is truly adrenergic is an interesting question. The cholinergic transfer of impulses in a sympathetic ganglion is, according to Bulbring and Burn (10), stimulated by small doses of adrenalin. We are studying at this time the possibilities of cholinergic dilator effects in human muscle and their relation to adrenalin. The effects of adrenalin in the sympathectomized muscle are also under investigation.

It is possible to speculate, in a general way, on the significance of our findings and those of other observers. We may assume then that circulating adrenalin in very small doses (and possibly also adrenalin or sympathin within the range of physiologic local release) effects a constriction of blood vessels of the skin and splanchnic area and has a dilating action upon muscle blood vessels. The dilator action is not sufficient for the requirements of a contracting muscle. However, a redistribution of circulating blood in favor of muscle will occur and be enhanced by an increased cardiac output. This may be advantageous if an emergency

develops requiring sudden and intense muscle action. During such an emergency, adrenalin if it reaches sufficient concentration will lose its dilator effect upon and finally will constrict muscle vessels. However, this will occur in inactive muscles only, while in the active hyperemic muscle the constrictor effect is vitiated. The dilator effect of locally released metabolites supervenes. Blood is shifted from inactive muscle groups to those in contraction. To substantiate this overall hypothesis and to fill in the gaps needs much further study of human vascular physiology. Such studies are also of clinical importance in view of the present tendency to perform sympathectomies rather liberally.

SUMMARY

1. Blood flow in the human forearm was studied by the plethysmograph method.

2. Injection of adrenalin in doses of 1–2 gamma into the brachial artery produces vasoconstriction.

3. Intra-arterial doses of 0.5 to 0.1 gamma adrenalin are apparently ineffective.

4. Doses of 0.05 to 0.0002 gamma adrenalin intra-arterially cause vasodilatation sometimes followed by vasoconstriction; 0.00002 gamma seems to be the threshold of vasodilator action. These observations are discussed.

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RENIN, HYPERTENSINOGEN, AND HYPERTENSINASE CONCENTRATION OF BLOOD OF DOGS DURING THE DEVELOPMENT OF HYPERTENSION BY CONSTRICTION OF THE RENAL ARTERY[1]

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The relation of the renal enzyme, renin, to the appearance and maintenance of hypertension in animals and man is not as yet clear. Many attempts have been made to demonstrate pressor or vasoconstrictor substances in the blood of hypertensive patients and of animals with experimental hypertension. A recent attempt has been made by Gregory, Ewing, Levin, and Ross (1) who have summarized the earlier literature. Although claims for the presence of pressor substances have been made, results have usually been negative, inconclusive, or not confirmed by other workers. Page (2) found that plasma from hypertensive patients caused vasoconstriction in the rabbit's ear. Plasma from normotensive individuals usually produced no vasoconstriction. He believed that the constriction was due to the presence of hypertensin (angiotonin) in the peripheral blood. Measurements by another method (3) in this laboratory have not shown demonstrable amounts of renin in the systemic blood of patients with chronic hypertension (4, 5), but occasionally small amounts have been detected in that of patients with acute hypertension (6). Few determinations of renin have been made in animals during the development of experimental hypertension. Page (7) reported the presence of renin in the renal venous blood of hypertensive dogs and commented that large amounts were found in one dog early in the course of malignant hypertension from cellophane perinephritis. Dell'Oro and Braun-Menéndez (8) found renin in the systemic blood of dogs in which the blood pressure was rising following the application of a clamp to the renal arteries. Since their animals were usually uremic from malignant hypertension, determinations could not be carried on for more than a few days after clamping the renal arteries due to death of the animals. The present study is similar to that of Dell'Oro and Braun-Menéndez, but the animals have been followed for longer periods and serial measurements of the hypertensinogen and hypertensinase as well as renin contents of the plasma have been performed.

METHODS. Ten dogs weighing 11 to 18 kgm. were operated upon aseptically under nembutal anesthesia to produce renal hypertension. Through a posterior incision the renal artery was exposed and clamped by means of a Goldblatt clamp (9) which was screwed down to occlude the vessel and then turned back about three-quarters of a turn. Usually a weak pulsation or a thrill could be felt distal to the clamp. In 3 dogs both renal arteries were clamped on the same

[1] This investigation was aided in part by a grant from the John and Mary R. Markle Foundation and from the Proctor Fund of the Harvard Medical School.

day. In the remainder, a period of 7 to 19 days was allowed between operations. Most of the animals received sulfadiazine, 0.5 gram per day, for 1 to 2 days before and after the operation.

Mean blood pressures were measured with a mercury manometer by direct puncture of the femoral artery at frequent intervals for at least 7 days before operation. After clamping the renal arteries, the blood pressure was measured every day or two as long as it continued to rise. At later periods, when blood pressures were taken at less frequent intervals, dogs were trained for several days in order to make readings comparable to previous ones.

Blood for the determination of renin, hypertensinogen, and hypertensinase was drawn from the femoral artery, or occasionally from the vein, into syringes containing at least 1 cc. of 3.8 per cent sodium citrate per 15 cc. of blood. Determinations on dog plasma were carried out in general as previously described for human and dog plasma (3, 10). Renin was determined on 8 to 10 cc. of plasma by a 2-hour incubation with an excess of beef hypertensinogen after the removal of hypertensinase by acidification to pH 2 for 2 hours at 4°C. in the presence of 9 per cent sodium chloride. Hypertensinogen was measured by a 10-minute incubation of 3 cc. of dog plasma in the presence of 100 cat units of hog renin. The amount of hypertensin formed in all determinations was assayed on cats as previously described (3). Comparative determinations were made on frozen plasmas, assuming that their hypertensinogen contents did not change under these conditions. Control measurements appear to indicate that this is a justtifiable assumption.

Nonprotein nitrogen was determined on frozen plasma samples saved from previous determinations.

Results. *Renin assay and renin content of normal dog plasma.* The recognition by assay of minute amounts of renin (usually 0.2 cat unit or less) is difficult by the method described due to the small atypical rises of pressure produced in the assay animal. In a sensitive assay preparation, typical and significant blood pressure rises can be obtained with only 0.1 cat unit, and in an insensitive preparation larger amounts of renin must be present to produce convincing blood pressure responses. When equivocal blood pressure rises have occurred, or when the blood pressure curve has been atypical for hypertensin, they have been recorded in the tables by the sign ±. In the 13 street dogs with normal blood pressure, assay of their plasma for renin yielded negative results in 6, equivocal (±) results in 4, and in the remaining 3, 0.2 to 0.4 cat unit per 10 cc. of plasma.

Renin and the course of hypertension. Pre-operative blood pressures in the series of dogs subsequently made hypertensive ranged from 98 to 132 mm. Hg. After clamping one renal artery only, moderate elevations of blood pressure occurred in only 2 of 6 animals. Five dogs, dying presumably of uremia 4 to 12 days after clamping the second renal artery, had some degree of hypertension (from 152 to 170 mm. Hg), sometimes occurring only terminally (table 1). The other 5 dogs survived the development of hypertension with levels between 155 and 192 mm. Hg (table 2). The blood pressure usually reached a peak in 1 to 2

weeks after the second renal artery was clamped and then fell over a period of weeks to a constant level about 30 mm. Hg above the pre-operative level (table 2). Two dogs, re-operated upon when they had remained at this level for some time, had enlarged arteries through the renal capsule as described by Elaut (11). In one of these animals the blood pressure rose 60 mm. Hg following ligation of these arteries on one kidney only.

In the hypertensive dogs renin appeared in the plasma within at least 1 or 2 days of clamping the second renal artery. In the majority of animals there was a rough parallelism between the rise of blood pressure and the increase in concentration of renin in the plasma. The amount of renin in the plasma reached a

TABLE 1

The effect of the production of renal hypertension on the renal pressor mechanism

DAYS	DOG 29				DOG 42					DOG 40					DOG 37			DOG 34			
	B.P., mm. Hg	Renin, CU*/10 cc.	H,** CU*/cc.	H,*** DU‡/cc.	B.P., mm. Hg	Renin, CU*/10 cc.	H,** CU*/cc.	H,*** DU‡/cc.	NPN, mgm. %	B.P., mm. Hg	Renin, CU*/10 cc.	H,** CU*/cc.	H,*** DU‡/cc.	NPN, mgm. %	B.P., mm. Hg	Renin, CU*/10 cc.	H,** CU*/cc.	B.P., mm. Hg	Renin, CU*/10 cc.	H,** CU*/cc.	NPN, mgm. %
Before	126	0	1.9[a]	1.4	122	±	2.9[a]	1.5		105	±	1.6[a]	2.0	44	105	0	2.1[a]	110	±	2.6[a]	
After	Clamp LRA				Clamp RRA					Clamp LRA								Clamp RRA			
1–2	130	0.3	2.0[a]	1.4						145	0.3	1.9[a]		68							
3–4	154	0.3	3.2[a]	1.2	122					122	0.3	2.4[a]	1.7					112			
5–6	140																				
7–8	140		1.3[b]							130	±	2.0[b]									
9–10					115	0.3			38									116			
After	Clamp RRA				Clamp LRA					Clamp RRA					Clamp LRA, RRA			Clamp LRA			
1	140		2.6[b]		124																
2	143	0	2.9[b]	1.2						138	0.5							106			
3	140		2.0[b]		160	±	3.3[a]	1.6		150								107			
4	Died				Died					170	1.2				160	2.2		104			
5										144		2.7[b]	1.7	200			0.3[a]				
11										Died					127	3.0	0.6[a]	152	0.8	2.4[a]	59
12															Died			Died			

RRA right renal artery, LRA left renal artery, * cat units, ‡ dog units, ** hypertensinogen, *** hypertensinase, a and b are determinations at the same time and therefore comparable.

maximum in 4 to 12 days (table 2) and then gradually diminished. In 2 dogs (dogs 36 and 39), renin was present in detectable amounts (0.4 to 0.6 cat unit per 10 cc. of plasma) for at least 90 to 100 days after operation. Its time of disappearance was variable in different animals. Despite the disappearance of detectable amounts of renin in the plasma, the blood pressure remained higher than before operation. After the blood pressure and renin levels had gradually decreased toward normal in dog 39, surgical reduction of the blood supply to the kidney by ligation of capsular vessels was followed by a rise of blood pressure and an increase of renin concentration in the plasma. In two instances (dogs 37 and 43), large amounts of renin were present when the blood pressure fell in the course of postoperative or terminal shock.

TABLE 2

The effect of the production of renal hypertension on the renal pressor mechanism

DAYS	DOG 33					DOG 35				DOG 36				DOG 39				DOG 43			
	B.P., mm. Hg	Renin, CU*/10 cc.	H,** CU*/cc.	H,*** DU‡/cc.	NPN, mgm. %	B.P., mm. Hg	Renin, CU*/10 cc.	H,** CU*/cc.	NPN, mgm. %	B.P., mm. Hg	Renin, CU*/10 cc.	H,** CU*/cc.	NPN, mgm. %	B.P., mm. Hg	Renin, CU*/10 cc.	H,** CU*/cc.	NPN, mgm. %	B.P., mm. Hg	Renin, CU*/10 cc.	H,** CU*/cc.	H,*** DU‡/cc.
Before	112 to 132	0			54	108 to 120		3.1^{a}	39	98 to 110	0.2	1.3^{a}	37	105 to 116	0	2.3^{a} 1.8^{b}		118 to 120	±	0.9^{a}	2.0^{a}
After	Clamp LRA													Clamp LRA				Clamp Branch RRA			
1–2	139													107							
14			1.8^{a}															124	0.2	1.8^{a,b}	
19	135	0	1.1^{b}	1.3																	
After	Clamp RRA					Clamp LRA, RRA				Clamp LRA, RRA				Clamp RRA				Clamp LRA			
1	129					120												130			
2	148	0.3	2.2^{a}			138				129	0.4	3.0^{a}		188		2.7^{a}		160	0.6	1.6	
3	154	0.4	2.2^{b}			160		2.5^{a}										174			
4										150	0.5		72	172	0.2		40	160	0.8		
5	135	0.6		1.5	91	172	0.8		85	143								162		1.9^{a}	
6	150																				
7	161	0.1				156				145				185	1.0	1.5^{b}	62	152		2.1^{a}	
8	156									150	0.3							152			
9	170	0.3	1.3^{b}		56	148		2.2^{a}		150				188				148			
10–12	190	0.2				175	1.3		45	140				160				140	0.4		
13–15	188	0.2	1.4^{b}	1.7		185				155				168							
16–20	172					148	0.5			149			34	168							
31–35						192	0.3		31					180	0.3	2.3^{a}	41				
36–40						152								170							
41–45	167	0.1				155	0							170				144			
51–100	149	0				145				125	0.6			152	0.4			140	0	1.3^{c,d,e}	1.7
101–150	Kill									130	0.2										
151–200						150	0	2.3^{a}		Kill				150		1.6^{b}					
238														135	0.3						
348						Died at								136	0						
431						520 days								130	±	1.4^{c}	44				
After														Tie CA				Clamp RRA			
1														155				160	0.4		
2														170				177		2.2^{c,e}	1.6
3														190	0.3		42	135	1.5		
4														182							
5														180	0.6		48				
6																		170	0.9	2.0^{c}	
7–9														185	0.4		49	165		2.1^{e}	1.9
10–12														180	1.0	1.0^{c}	50	164		2.4^{d}	2.3
13–15														176	1.2	0.8^{c}	43		0.4		
16–20																				2.7^{d}	2.2
21–25														180	1.5	0.6^{c}	47				
26–30														182	0.5	0.6^{c}	45	142			
90–100														Died suddenly				148	±		
														at 28 days				Kill			

RRA right renal artery, LRA left renal artery, CA capsular arteries, * cat units, ‡ dog units, ** hypertensinogen, *** hypertensinase, a, b, c, d, e are determinations at the same time and therefore comparable.

Hypertensinogen. The rise of blood pressure and of renin concentration in plasma was frequently unaccompanied by any significant change in the concentration of hypertensinogen. In 2 dogs (dogs 37 and 39), a decrease of hyper-

tensinogen concentration was associated with high concentrations of renin in the plasma. With the exception of dog 37, no animal dying in uremia showed changes of hypertensinogen concentration prior to death. Three dogs (dogs 29, 43, and 36), showed increases in the concentration of hypertensinogen after constricting the renal artery.

Hypertensinase. The hypertensinase content of the plasma was determined before and after operation in 5 instances. No definite or consistent changes were evident. If large amounts of renin are present in the plasma, falsely low values may be obtained due to the formation of hypertensin during the incubation of the plasma sample.

Nonprotein nitrogen. In 4 of 5 dogs surviving the operation for more than 12 days, the nonprotein nitrogen of plasma was found to be increased before or at the time of the maximum renin level in the plasma (the rise being 22 to 46 mgm. per cent). Measurements were not frequent enough to determine the duration of the rise. In dog 39, relatively large amounts of renin appeared without significant change in the nonprotein nitrogen level.

Discussion. The precise mechanism by which the blood pressure rises following constriction of the renal artery has been the subject of considerable investigation and controversy, a variety of substances having been suspected but not proven as the causative agent. The renin system has received particular attention in recent years, especially in acute experiments but little in the developmental and chronic stages of experimental hypertension. Dell'Oro and Braun-Menéndez (8) have followed renin levels in the plasma for a few days after constricting the renal artery and have obtained findings similar to ours.

The observations in this report shed a certain amount of light on the relation of the renin system to the development of experimental renal hypertension but are not definitive. It is apparent that there is a gradual increase in the concentration of renin in the blood over a period of days. The greatest amounts of renin detected during the development of hypertension (table 2) were 0.6 to 1.5 cat units per 10 cc. of plasma. Similar blood pressure elevations and renin concentrations may be produced with infusions of 0.5 to 1.0 cat unit per minute of hog renin (12). Interestingly enough, at the time of maximal ischemia of the kidney, i.e., just after the Goldblatt clamp is applied, the renin concentration is not at a maximum. Subsequently, at a time when the renin concentration has reached its peak and begun to decline, the blood pressure continues to remain elevated. Despite the persistence of some degree of hypertension, the renin concentration gradually falls over a period of weeks or months until it is no longer detectable. In dogs with hypertension of months' or years' duration, renin has not been detectable in the systemic blood (unpublished observations). Nephrectomy, however, produces a prompt fall of blood pressure within a few hours (13, 14, 15, 16, 17). Although there is a rough parallelism between the concentration of renin in the blood and the appearance of hypertension, there is no apparent relationship between the two in the chronic stages by the technique utilized in this communication.

In our series of dogs, there was no consistent change in the concentration of

hypertensinogen in the plasma as hypertension developed. In two instances, it became diminished, and in three it increased. The decrease was associated in each case with high concentrations of renin. Nephrectomy is followed by an increased concentration of hypertensinogen, the increase having been attributed to the absence of circulating renin (18, 19). The mechanism of the increase in our animals whose plasma contained more renin than normal must have been on some other basis, the nature of which is not immediately apparent.

No definite or consistent changes in the concentration of hypertensinase in the plasma of these dogs was evident.

We believe that in the light of present knowledge, no single interpretation of these observations is warranted. The following possibilities suggest themselves:

1. It is possible that the renin system has nothing to do with the development and maintenance of experimental renal hypertension and that its production is determined by autolysis of kidney tissue. It will be observed that in 4 of the 5 animals in which serial determinations of nonprotein nitrogen were recorded, some elevation occurred during the development of the hypertension. In the fifth dog (dog 39), blood pressure and renin concentration became elevated without significant changes in the nonprotein nitrogen values following ligation of the collateral blood supply in the renal capsule. Although Goldblatt *et al.* (20) observed no elevation of nonprotein nitrogen in their dogs with hypertension from constriction of the renal artery, others have observed transient slight elevations of nonprotein nitrogen (21, 22) or blood urea nitrogen (11, 23). Most reports indicate minor decreases of renal function for short periods after mild to moderate constriction of the renal artery, but such findings are transient for there is a rapid return to normal of phenolsulfonephthalein, urea, inulin, phenol red, and diodrast clearances (20, 24, 25, 26, 27, 28).

2. Renin may initiate the hypertension and some other pressor mechanism may subsequently take over the function of its maintenance. This has been suggested by Reed, Sapirstein, Southard, and Ogden (29, 30) on the basis of experience with sympatholytic drugs. While yohimbine and F-883 caused no fall of blood pressure in early renal hypertension, a fall of blood pressure was produced in the chronic stages. They concluded that in early renal hypertension, the blood pressure rise was mediated by a renal humoral pressor mechanism unaffected by sympatholytic agents, whereas in late renal hypertension the blood pressure was elevated by a sympathetic neural pressor system capable of being blocked by these drugs.

3. Methods may be too insensitive to detect renin in chronic hypertension. The concentration present during the acute phase of hypertension may be in excessive, easily measurable amounts while the ischemia is at its height. Such excessive amounts of renin in the blood may diminish after the elevation of blood pressure has relieved to some extent the ischemia, or after the opposite kidney, if untouched, has had time to take over the function of the clamped kidney through the medium of compensatory hypertrophy, or after the collateral circulation through the capsule of the clamped kidney has begun to develop. The amount of renin which is necessary to maintain the blood pressure at a level adequate for

the circulatory needs of the kidney may be too small to be detectable by the method employed in our study. No information is available regarding the minimal amount of renin that is required to elevate blood pressure over a period of time. The effects on the blood pressure of a continuous infusion of minute amounts of homologous renin to animals over a period of days has not as yet been reported.

Which, if any, of these interpretations is correct cannot be stated at this time because as much evidence for as against each one can be offered. While the data do not support the hypothesis that the renin-hypertensinogen-hypertensin system is the mediator of the hypertension following the application of a Goldblatt clamp, it should be emphasized that the evidence presented is by no means conclusive in excluding such a possibility.

SUMMARY

1. During the development of experimental renal hypertension in dogs, renin was detected in the plasma.

2. The renin level reached a maximum about one week after both renal arteries were constricted and then gradually diminished.

3. A rise in nonprotein nitrogen was often, but not necessarily, present when the renin concentration of plasma was increased.

4. With the development of hypertension, the hypertensinogen content of the plasma remained unchanged or increased somewhat unless the amount of renin became excessive, in which case the hypertensinogen concentration decreased.

5. No significant changes in the hypertensinase content of the plasma were noted.

6. The significance of these observations is discussed.

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RENIN CONTENT OF RENAL VENOUS BLOOD OF NORMAL AND HYPERTENSIVE PATIENTS AT REST[1]

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Previous studies (1, 2, 3, 14) have failed to reveal the presence of renin in the systemic blood of patients with chronic hypertension. Renin is known to be produced by the kidney and liberated into the systemic circulation by way of the renal vein (4, 5, 6). Since it has been suspected but not proven to be the humoral agent responsible for the elevation of blood pressure in human hypertension, a study has been made of the renin concentration of renal venous blood in a group of normal and hypertensive patients.

METHODS. Twelve patients with hypertension from different causes and of varying degrees of severity (see table 1) and 12 patients with normal blood pressures were chosen for study. Only one of the patients had evidence of congestive heart failure at the time of study. The patients were transferred to the fluoroscopy room in a fasting state where they lay on the fluoroscopic table which was covered with a thick mat of sponge rubber to insure their physical comfort. Pillows were used judiciously under head and shoulders. An 8-F or 9-F radio-opaque venous catheter, 100 cm. long and with the hole directly on the tip, was introduced into a vein of the forearm as described by Cournand and Ranges (7). Novocaine without adrenalin was used as a local anesthetic. The catheter was directed fluoroscopically into the renal vein[2] as described by Warren *et al.* (8). Its position was checked before and after withdrawal of the blood sample. In each instance, it was observed to leave the course of the inferior vena cava opposite the second lumbar vertebra (see fig. 1), and the color of the renal venous blood was observed to be more red than that taken from other parts of the venous system. Thirty to 40 cc. of blood were withdrawn from the renal vein into syringes containing one or more cubic centimeters of 3.8 per cent sodium citrate per 15 cc. of blood. A similar amount of blood was occasionally withdrawn as a control from either the superior vena cava, right auricle, right ventricle, or pulmonary artery. The renin titer of the blood was assayed as previously described (9).

RESULTS. The findings in this study are summarized in table 1. Seven of

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[2] The authors are indebted to Dr. Stanley E. Bradley for teaching them the technique of venous catheterization of the heart and renal vein and for collecting samples of renal venous blood in 4 of the patients reported in this study.

TABLE 1

Renin content of renal venous blood

PATIENTS WITH NORMAL BLOOD PRESSURE					PATIENTS WITH HYPERTENSION				
No.	Patient	Diagnosis	Blood pressure	Renin cat units per 10 cc. plasma	No.	Patient	Diagnosis	Blood pressure	Renin cat units per 10 cc. plasma
			mm. Hg					*mm.* Hg	
1.	L. S.	Asthma	118/84	0.3	1.	F. M.	Essential hypertension	222/146	0.9
2.	M. H.	Dermatitis	112/82	0.3	2.	W. W.	Chronic nephritis; calcification of kidneys	198/114	0.7
3.	S. O.	Pneumonia (convalescent)	120/80	0.3	3.	J. H.	Essential hypertension	186/134	0.5
4.	H. R.	Duodenal ulcer	90/62	0.3	4.	J. S	Chronic pyelonephritis, uremia	200/116	0.5
5.	B. F.	Pulmono-cardiac disease	110/75	0.3	5.	J. W.	Coarctation of aorta	216/141	0.5 Right 0.2 Left
6.	R. F.	Rheumatic fever (convalescent)	120/74	0.3	6.	J. S.	Nephrotic syndrome	167/92	0.3
7.	M. T.	Lues (treated)	105/70	0.2	7.	S. H.	Chronic glomerulonephritis	139/102	0.2
8.	F. B.	Emphysema	126/82	±	8.	M. S.	Acute glomerulonephritis	170/100	0
9.	H. G.	Spontaneous pneumothorax	128/88	0	9.	C. F.	Unilateral renal disease, left	170/110	0 Left 0 Right
10.	H. P.	Lues (penicillin)	106/66	0	10.	W. P.	Malignant hypertension	200/140	0
11.	G. J.	Peptic ulcer	114/80	0	11.	E. W	Malignant hypertension, cardiac failure	196/142	0
12.	W. W.	Lues (treated)	113/75	0	12.	J. P.	Chronic pyelonephritis, left	190/120	0 Left 0 Right

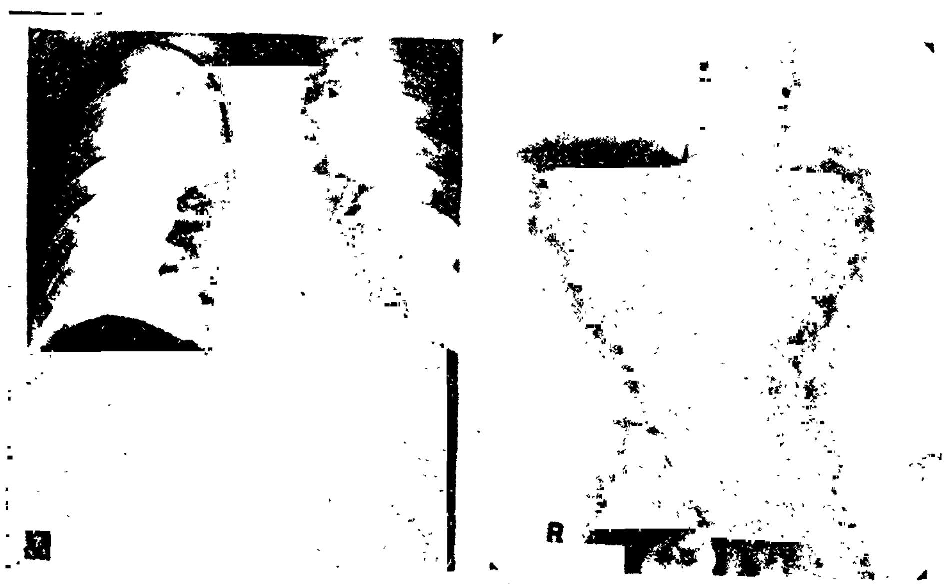

Fig. 1. X-ray showing venous catheter passing through axillary and subclavian vein, superior vena cava and right auricle (left) into inferior vena cava and right renal vein opposite the second lumbar vertebra (right).

the 12 hypertensive patients and 7 of the 12 patients with normal blood pressures had small but definite amounts of renin, while in the remainder of each group no renin or equivocal amounts of renin were present.

Discussion. The data indicate that under the conditions of the experiments, the renal venous blood of normal individuals contained about as much renin with the same frequency as did the renal venous blood of hypertensive individuals. The amount of renin in the hypertensives might seem to have been a little greater, but in considering the errors of the assay method such differences were not considered significant.

In the hypertensive group, there was no relation between the severity or etiology of the hypertension and the concentration of renin in the renal venous blood.

Renin is detectable in the systemic blood of animals and sometimes in man during the early phase of hypertension when the blood pressure is rising (10, 11, 12, 13). After a period of time, it no longer remains detectable in the systemic blood despite the persistence of the hypertension (1, 3, 12). This study reveals its presence in minute amounts in renal venous blood in about the same concentration as in normal individuals. Such findings do not support the hypothesis that renin is the humoral substance responsible for human hypertension.

On the other hand, these observations are not conclusive in excluding such a possibility. Although the patients were at rest and although their blood pressure and pulse rates were unchanged in several instances during the withdrawal of the renal venous samples, they were not in a strictly "basal" state. Introduction of a catheter into the renal vein may well be sufficient to liberate at times a small amount of renin by the kidney. The possibility remains, therefore, that the concentrations of renin observed by us were produced by such factors. Since many physiological substances are active in exceedingly minute amounts, it is possible that amounts, undetectable by the method employed, were acting day after day to maintain an elevated blood pressure in the hypertensive patients. We do not believe that either of the above interpretations can be proven or disproven by the techniques utilized in this study.

SUMMARY

1. Renin was detected in the renal venous blood in approximately the same amount and with the same frequency in a group of 12 hypertensive patients and in a group of 12 patients with normal blood pressures at rest.

2. The significance of these findings in relation to the etiology of human hypertension is discussed.

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ALVEOLAR AIR DURING SIMULATED FLIGHTS TO HIGH ALTITUDES[1]

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Heretofore, practically all extensive measurements of alveolar gas composition have been made on samples intermittently obtained from forcible expirations. Such methods require a certain amount of co-operation on the part of both subject and operator, and are bound to interfere with the normal breathing pattern. The sampling and analyzing device described by Rahn, Mohney, Otis, and Fenn (1946) makes possible the continuous sampling and minute to minute recording of alveolar air of a subject quietly breathing from a face mask. The purpose of the present investigation was to obtain by this method extensive data concerning the respiratory behavior of several thoroughly trained subjects[2] breathing under normal resting conditions and under a variety of experimental conditions, particularly during acute exposure to the simulated altitudes of 12, 16, 18, 20, and 22 thousand feet.

Outline of Flights. Series I–V. All experiments were begun at 9 a.m. or 2 p.m. The subject was seated in the high altitude chamber during all measurements and breathed air through a face mask from a Pioneer demand valve. After a twenty minute period at ground level (540 ft., elevation above sea level), during which control measurements were made, ascent to the desired altitude occurred at a rate of 4500 feet per minute. An observer breathing oxygen accompanied the subject. Measurements were resumed as soon as the desired altitude was reached. Experiments were one hour in length at the 12, 16, and 18 thousand foot altitudes, 45 minutes at 20,000 feet and 30 minutes at 22,000 feet. Each subject was exposed to each altitude from lowest to highest in turn with at least a one day interval between successive exposures. The same eight subjects participated in all these experiments. Series III A was identical with series III.

Series VI and VII. In addition to the "normal" flights (series I to V) each of the eight subjects also participated in similar one-hour flights to 12,000 and 18,000 feet which were preceded by the ingestion of 100 grams of glucose in 200 cc. of water just before the ascent.

Series VIII. CO_2 inhalation at 16,000 feet: in this series four subjects breathed 6.0 per cent CO_2 in air for a 15 minute period. The experimental

[1] Work done under contract recommended by Committee on Medical Research between the Office of Scientific Research and Development and the University of Rochester.

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period was preceded by a 10-minute control period and followed by a 10-minute recovery period, both at 16,000 feet. No performance tests were administered during these experiments.

Series IX and X. Muscular work at ground level and 16,000 feet. For the muscular work, the sitting subject pushed his feet alternately against pedals constructed from flat pieces of spring steel at a rate of 30 times a minute for each foot. The mechanical work required for this task was calculated to be 49.4 kilogram-meters per minute. The period of actual work was 10 minutes in length. It was preceded by a control period and followed by a recovery period of the same duration.

METHODS. *Alveolar gas composition* was read and recorded each minute by use of the continuous method referred to above. In addition, an end-expiratory Haldane-Priestley sample was obtained every ten minutes and subsequently analyzed by the Haldane method or by the same meters used for the continuous method. The discrepancy between the conventional and the continuous methods was thus obtained.

Frequency of breathing was recorded outside the chamber on a paper tape which moved at a known constant rate. The slight negative pressure developed in the mask during each inspiration was made to close a relay circuit by means of a switch constructed from a sensitive aneroid barometer. Closure of the circuit activated an electromagnet to which was attached the recording pen, which thus made a deflection during each inspiration.

Ventilation volume was recorded on the same tape by another electro-magnetically operated pen. The subject expired through a gas meter so fitted with electrical contacts that its rotating arm briefly closed the pen circuit once for each 500 cc. of gas passing through the meter. The temperature was read from a thermometer inserted into the outlet of the meter.

Percentage saturation of the arterial blood was read and recorded each minute by means of the Millikan oximeter using the single scale circuit with automatic adjustment for ear thickness (Millikan, 1942).

Heart rates were recorded for one minute out of each ten by means of an electrocardiotachometer.

Performance was measured every ten minutes by the hand steadiness test previously described (Rahn, Otis et al.).

RESULTS. *Normal runs* (*series I through V*) *and glucose runs* (*series VI and VII*). The data obtained are summarized in tables 1–3. Respiratory data are averages of the minute to minute readings of each five-minute period indicated. Each value, therefore, is based on five measurements on each subject or a total of 40 separate readings, except in series III A, in which only six subjects participated and in series V in which one subject lost consciousness after ten minutes at altitude. Heart rates and steadiness test scores are indicated at ten minute intervals as explained in the legend accompanying the tables.

Carbon dioxide inhalation experiments (series VIII) and exercise runs (series IX and X) are summarized in tables 3 and 4, respectively, which are similar to the previous tables except that the respiratory data are averaged for 2-minutes in-

TABLE 1

	TIME—MINUTES															
	5	10	15	20	5	10	15	20	25	30	35	40	45	50	55	60
	Ground Level, B = 746				12,000 Ft. B = 483, Series I											
1. Alveolar pO_2	102.2	100.8	101.5	101.0	54.5	52.1	51.2	51.0	50.0	50.4	49.5	51.4	51.2	50.9	49.4	50.7
2. Alveolar pCO_2	37.0	37.2	36.8	37.6	35.1	35.4	35.1	35.1	35.2	34.9	35.3	34.5	34.3	34.3	35.1	34.0
3. Alveolar R.Q.	0.82	0.80	0.81	0.81	0.95	0.89	0.86	0.85	0.83	0.85	0.83	0.83	0.83	0.83	0.81	0.82
4. Ventilation l./min.	8.80	9.10	9.02	8.85	10.60	9.45	9.82	9.48	9.42	9.50	9.53	9.66	9.72	9.61	9.80	9.77
5. Breaths/min.	13	14	14	13	16	14	14	13	14	14	14	14	14	14	15	14
6. % HbO_2 (Calc.)					89	88	87	87	86	87	86	88	87	87	86	87
7. % HbO_2 (Oximeter)				98	89	87	85	86	85	85	84	85	85	85	85	85
8. Ml. O_2/min. (Calc.)	314	334	327	324	312	302	327	319	321	313	326	325	326	317	336	331
9. Heart Rate % Normal			*(82)	100		113		115		113		106		104		99
10. Stead. Test % Normal			*(386)	100		129		157		104		139		128		113
	Ground Level, B = 751				16,000 Ft. B = 412, Series II											
1. Alveolar pO_2	107.1	107.9	107.0	106.1	46.1	45.6	44.4	44.8	46.1	45.4	44.4	43.2	44.2	44.4	43.5	44.2
2. Alveolar pCO_2	35.1	34.6	34.9	34.9	32.4	30.8	30.8	30.0	28.5	28.6	28.9	29.4	28.6	28.2	27.9	28.0
3. Alveolar R.Q.	0.86	0.87	0.83	0.83	1.11	1.03	0.99	0.96	0.98	0.95	0.91	0.87	0.89	0.87	0.82	0.86
4. Ventilation l./min.	8.69	8.69	8.61	8.25	9.50	9.32	8.98	9.14	9.40	9.74	9.36	8.79	9.76	9.39	8.90	10.50
5. Breaths/min.	11	12	12	13	12	12	11	11	11	13	12	12	12	12	13	11
6. % HbO_2 (Calc.)					85	85	84	84	85	85	84	83	84	85	84	84
7. % HbO_2 (Oximeter)					82	80	78	79	79	79	80	78	80	81	79	82
8. Ml. O_2/min. (Calc.)	301	281	297	275	233	225	244	244	236	248	248	250	270	254	258	306
9. Heart Rate % Normal			*(81)	100		111		110		103		103		101		105
10. Stead. Test % Normal			*(326)	100		91		154		265		210		166		196

	Ground Level, B = 751				18,000 ft. B = 379, Series III											
1. Alveolar pO_2	108.2	105.3	105.5	107.1	44.1	43.1	41.8	41.3	41.5	40.1	41.1	40.8	40.2	39.8	40.3	41.1
2. Alveolar pCO_2	34.6	34.9	34.8	35.0	28.8	28.4	28.0	27.3	26.6	27.0	26.7	26.2	25.7	26.2	25.4	25.1
3. Alveolar R.Q.	0.88	0.82	0.82	0.86	1.23	1.15	1.06	1.02	0.99	0.94	0.93	0.93	0.90	0.88	0.88	0.90
4. Ventilation l./min.	8.55	8.45	8.49	8.42	11.38	10.90	11.02	11.24	11.24	11.06	11.10	11.73	11.43	10.66	10.63	10.83
5. Breaths/min.	12	11	12	12	11	11	11	12	12	11	11	11	12	12	12	11
6. % HbO_2 (Calc.)					84	84	82	82	82	81	81	82	81	81	82	83
7. % HbO_2 (Oximeter)					80	79	77	78	75	75	76	76	77	77	76	78
8. Ml. O_2/min. (Calc.)	275	304	294	279	246	246	266	271	271	292	292	307	296	282	272	275
9. Heart Rate % Normal			*(82)	100		107		109		108		111		108		104
10. Stead. Test % Normal			*(301)	100		670		372		405		442		458		392

Each column contains average values on all subjects during the indicated period of time. The meaning of the rows is as follows:

1. The alveolar oxygen tension in millimeters of mercury.
2. The alveolar carbon dioxide tension in millimeters of mercury.
3. The alveolar respiratory quotient as calculated by equation (6) from Fenn, Rahn, and Otis, 1946.
4. The ventilation rate in liters per minute calculated at B.T.P.S. (body temperature, ambient pressure, saturated).
5. The frequency of breathing per minute.
6. The percentage saturation of the arterial blood as estimated from the alveolar pCO_2 and pO_2 with the nomogram of L. J. Henderson (1928).
7. The percentage saturation of arterial blood as indicated by the Millikan oximeter.
8. The oxygen consumption in milliliters per minute S.T.P. calculated from the relationship

$$X_o = \frac{Va \times pC}{0.864 \times Q}$$

Where Va = alveolar ventilation in liters per minute (B.T.P.S.)
pC = alveolar pCO_2 in mm. Hg
Q = alveolar respiratory quotient

$$0.864 = \frac{310}{273} \times \frac{760}{1000}$$

A constant dead space of 210 cc. was assumed in computing Va from the total ventilation (150 cc. personal dead space plus 60 cc. apparatus dead space).

9. The heart rate as per cent of the resting heart rate at ground level. These values are averages based on several measurements during each indicated 10-minute period. The average value for the control rate in beats per minute is the figure in parenthesis indicated by an asterisk.

10. Steadiness test score as per cent of the average value at ground level. The actual value of the ground level score expressed as time of contact in 1/240 second out of a 30-second test period is the value in parentheses indicated by an asterisk. The steadiness test was administered every 10 minutes and so appears in alternate 5-minute periods.

Each figure in series I–VII represents an average of (8 × 5) 40 readings. The R.Q. is the average taken from the original data and may differ slightly when computed from average oxygen and carbon dioxide values in these tables, due to dropping of decimals and rounding off.

TABLE 2

	TIME—MINUTES															
	5	10	15	20	5	10	15	20	25	30	35	40	45	50	55	60
	Ground Level, B = 746				18,000 Ft. B = 379, Series III-A											
1. Alveolar pO_2	100.8	103.1	102.3	104.4	43.4	42.3	40.8	39.8	40.5	40.6	39.7	38.5	39.6	38.9	38.6	36.6
2. Alveolar pCO_2	37.4	36.4	36.5	36.3	29.3	27.8	27.7	28.5	26.8	26.1	27.1	28.3	27.1	26.6	27.0	28.3
3. Alveolar R.Q.	0.79	0.82	0.81	0.84	1.22	1.13	1.00	0.99	0.94	0.94	0.95	1.02	0.96	0.89	0.87	0.87
4. Ventilation l./min.	8.99	9.26	9.24	8.93	13.51	13.11	12.96	12.03	12.33	11.02	12.06	11.35	12.18	10.68	11.06	10.38
5. Breaths/min.	15	15	14	15	12	12	14	14	14	13	15	15	14	13	14	14
6. % HbO_2 (Calc.)					82	83	81	80	82	82	81	80	80	80	79	77
7. % HbO_2 (Oximeter)					79	77	75	73	73	75	74	71	73	72	73	70
8. Ml. O_2/min. (Calc.)	318	312	330	287	306	302	334	304	312	267	292	237	303	276	293	282
9. Heart Rate % Normal			*(88)	100		112		111		113		104		99		107
	Ground Level, B = 745				20,000 Ft. B = 349, Series IV											
1. Alveolar pO_2	104.1	104.2	100.7	104.8	39.8	37.6	36.8	36.7	36.4	36.4	36.8	37.9	37.0			
2. Alveolar pCO_2	36.5	35.1	37.0	35.5	27.1	26.6	26.3	24.4	25.6	24.4	24.5	23.4	23.2			
3. Alveolar R.Q.	0.85	0.85	0.79	0.85	1.21	1.03	0.97	0.94	0.93	0.94	0.89	0.89	0.84			
4. Ventilation l./min.	8.30	8.71	8.12	8.40	13.77	12.36	12.66	11.76	12.23	12.46	12.60	13.44	12.56			
5. Breaths/min.	13	12	11	11	11	11	11	11	12	12	12	13	13			
6. % HbO_2 (Calc.)					81	79	78	78	78	80	79	80	79			
7. % HbO_2 (Oximeter)					78	74	74	73	72	74	75	77	76			
8. Ml. O_2/min. (Calc.)	278	297	315	296	298	301	325	284	310	299	321	326	315			
9. Heart Rate % Normal			*(84)	100		124		112		117		107				
10. Stead. Test % Normal			*(362)	100		365		651		928		775				
	Ground Level, B = 747				22,000 Ft. B = 321, Series V											
1. Alveolar pO_2	105.0	103.9	102.9	103.3	36.1	34.0	33.6	32.7	31.8	32.0						
2. Alveolar pCO_2	34.7	35.2	35.5	35.3	24.4	25.1	23.8	24.6	24.1	23.5						
3. Alveolar R.Q.	0.83	0.81	0.82	0.81	1.26	1.11	1.05	1.01	0.95	0.95						
4. Ventilation l./min.	8.72	8.18	8.57	8.64	16.79	14.61	15.82	15.21	14.15	15.31						
5. Breaths/min.	10	11	11	11	14	12	14	14	14	15						
6. % HbO_2 (Calc.)					78	75	75	73	72	72						
7. % HcO_2 (Oximeter)					73	68	69	66	63	64						
8. Ml. O_2/min. (Calc.)	321	296	315	320	311	317	338	347	330	359						
9. Heart Rate % Normal			*(88)	100		131		126		124						
10. Stead. Test % Normal			*(365)	100		354		1217		1702						

TABLE 3

	TIME—MINUTES															
	5	10	15	20	5	10	15	20	25	30	35	40	45	50	55	60
	Ground Level, B = 749				12,000 Ft. B = 483, Series VI—Glucose											
1. Alveolar pO_2	101.6	101.5	103.8	102.9	54.4	52.8	52.6	52.6	52.1	52.7	51.7	52.1	53.2	52.8	52.7	52.7
2. Alveolar pCO_2	37.9	38.5	36.5	37.4	34.6	35.2	34.9	34.5	34.7	34.8	34.9	34.4	34.3	34.0	34.0	34.2
3. Alveolar R.Q.	0.83	0.85	0.84	0.83	0.95	0.91	0.90	0.89	0.89	0.87	0.87	0.87	0.90	0.88	0.88	0.89
4. Ventilation l./min.	7.72	8.12	8.09	8.34	9.34	9.06	9.37	8.96	9.29	8.96	8.91	8.97	9.33	8.91	9.32	8.67
5. Breaths/min.	12	13	12	12	12	12	13	12	13	12	13	12	12	12	12	11
6. % HbO_2 (Calc.)					89	89	88	89	88	89	88	88	89	89	89	89
7. % HbO_2 (Oximeter)					89	89	88	86	86	86	87	84	86	86	87	87
8. Ml. O_2/min. (Calc.)	276	288	281	304	289	294	300	290	298	299	289	300	301	287	305	283
9. Heart Rate % Normal			*(86)	100		110		109		104		107		109		102
10. Stead. Test % Normal			*(251)	100		119		119		98		115		143		137
	Ground Level, B = 749				18,000 Ft. B = 379, Series VII—Glucose											
1. Alveolar pO_2	103.0	102.0	101.5	102.8	41.7	39.7	39.2	38.6	38.8	38.9	37.7	38.1	38.0	38.5	38.1	37.6
2. Alveolar pCO_2	37.6	37.8	38.3	37.5	29.8	30.5	28.4	30.5	29.9	30.1	30.2	29.6	29.4	30.3	29.2	29.4
3. Alveolar R.Q.	0.84	0.82	0.82	0.83	1.11	1.04	1.04	1.00	0.98	0.99	0.96	0.95	0.93	0.94	0.93	0.95
4. Ventilation l./min.	8.40	7.66	8.39	8.02	12.45	11.23	12.13	11.13	11.10	11.00	11.26	11.26	11.53	11.00	11.50	11.32
5. Breaths/min.	11	11	11	11	12	12	14	12	11	12	16	13	15	15	13	16
6. % HbO_2 (Calc.)					82	80	79	79	79	79	77	78	78	79	78	79
7. % HbO_2 (Oximeter)					82	81	79	78	78	79	78	77	78	78	79	79
8. Ml. O_2/min. (Calc.)	315	287	329	298	310	296	293	304	311	299	386	308	305	291	320	284
9. Heart Rate % Normal			*(85)	100		125		122		122		121		120		121
10. Stead. Test % Normal			*(324)	100				386		618		522		373		320

TABLE 3—*Concluded*

	TIME—MINUTES																	
	2	4	6	8	10	12	14	16	18	20	22	24	25	27	29	31	33	35
	Series VIII.—6% CO_2 at 16,000 Feet																	
	Normal					6% CO_2								Return to Normal				
1. Alveolar pO_2	49.0	47.8	47.2	47.9	46.4	46.1	47.4	47.7	48.1	48.9	49.3	49.2	49.4	47.9	46.4	46.2	45.4	45.9
2. Alveolar pCO_2	32.0	30.9	30.9	30.0	30.5	36.3	40.0	40.2	41.4	41.2	41.4	41.5	41.5	35.7	32.2	31.9	31.9	31.7
3. Alveolar R.Q.	1.22	1.13	1.11	1.09	1.05	0.89	0.72	0.77	0.82	0.82	0.86	0.85	0.85	1.39	1.12	1.13	1.07	1.08
4. Ventilation l./min.	13.87	12.36	12.73	13.87	12.14	12.79	13.80	15.16	15.10	15.45	15.09	15.96	15.37	14.95	12.56	12.07	12.10	11.83
5. Breaths/min.	11	12	11	10	10	11	12	14	12	13	14	14	13	13	12	11	11	10
6. Heart Rate					87								79					83
7. % HbO_2 (Calc.)	88	87	86	87	85	84	84	84	83	84	85	85	85	86	86	85	85	85
8. Oximeter	87	86	85	86	84	84	85	85	85	84	84	84	84	85	82	82	81	81

stead of for 5-minute periods. Grand averages for each 10-minute period are also shown in table 4.

DISCUSSION. 1. *Alveolar Gas Composition and Ventilation at Altitude.* The data presented for series I through V represent respiratory behavior typical of healthy, young, well trained male subjects during acute exposure to simulated altitudes. It should be emphasized that all subjects were thoroughly indoctrinated beforehand, and that they worked as a full-time laboratory team as subjects and technicians during the course of the experiments. They were, therefore, largely free of the apprehension that sometimes occurs in the case of individuals who are used only occasionally as subjects.

An important feature of the present data, made conveniently possible by the method of sampling, is that the time course of changes in alveolar gas composition is traced throughout the period of exposure to altitude. In this respect these data supplement the standard alveolar curves of Boothby (1944), which are based on values obtained at 10 and 15 minutes at altitude. Furthermore his subjects went successively to higher altitudes on the same run, thus partially adapting at each level. These time changes are strikingly reflected by the values for the alveolar respiratory quotient. Upon arrival at altitude the R.Q. is increased above normal owing to the hyperventilation resulting from the stimulus of anoxia. This hyperventilation is accomplished largely by increased tidal volume until 22,000 feet where the rate also increases. With time the CO_2 stores of the body are depleted and the R.Q. gradually drops back toward normal indicating the approach of a new steady state. Related to this changing R.Q. are the changes in alveolar gas tensions. The pO_2, relatively high at first, gradually drops with the R.Q. even though the pCO_2 may remain approximately constant or become considerably diminished. The percentage saturation of the arterial blood also tends to drop in parallel with the pO_2.

These significant facts are conveniently summarized graphically in figure 1 by use of the CO_2-O_2 diagram introduced by Fenn, Rahn and Otis (1946). Since the family of R.Q. lines have the same slopes but a different intercept at different altitudes, the various altitudes involved may be represented on a single set of R.Q. lines by use of a multiple scale of abscissae. This has been done for convenience in figure 1. In addition, lines of constant alveolar ventilation have been drawn for the values of 6, 8, 10, and 12 liters per minute on the assumption that the O_2 intake is constant. The locations of these lines were determined from equation 19 of our previous report (Fenn, Rahn and Otis, 1946). X_0 in this case, was taken as 300 cc. per minute, the average calculated oxygen consumption during the flights represented on the diagram. This seemed justifiable because no consistent effects of the altitudes on oxygen consumption can be observed in the data.

Several pertinent facts are emphasized by this mode of presentation. One may think of the immediate respiratory response to a given altitude as being represented by a jump from the ground level alveolar point on the diagram to the initial altitude point. During the first five minutes at any altitude the alveolar ventilation is greater than during the subsequent periods. This reflects the

TABLE 4

	TIME—MINUTES														
	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
	Series IX.—Work at Ground Level														
	Resting					Work B = 746					Recovery				
1. Alveolar pO_2	101.3	101.5	101.7	102.2	100.1	94.5	96.9	97.6	98.7	98.5	102.8	101.9	102.4	102.0	102.8
2. Alveolar pCO_2	37.7	37.8	38.0	37.3	38.5	41.7	41.4	41.3	41.3	41.0	37.7	38.0	37.5	37.5	36.7
3. Alveolar R.Q.	0.83	0.82	0.86	0.85	0.83	0.79	0.83	0.83	0.86	0.84	0.87	0.86	0.85	0.84	0.84
4. Ventilation l./min.	9.12	8.72	8.63	8.79	9.06	12.18	13.83	13.99	14.85	13.94	10.81	8.85	8.89	8.81	8.76
5. Breaths/min.	12	12	12	12	12	15	14	15	15	16	13	13	13	13	13
6. Ml. O_2/min. (Calc.)	349	332	314	320	353	548	632	622	661	595	407	315	316	315	306
7. Heart Rate					84					92					87
	Series X.—Work at 16,000 Feet														
	Resting					Work B = 412					Recovery				
1. Alveolar pO_2	46.6	44.6	44.2	43.4	43.0	41.0	40.6	40.1	39.6	39.8	41.6	41.3	41.6	41.0	40.8
2. Alveolar pCO_2	33.6	33.8	33.6	33.3	33.4	35.3	35.2	35.3	35.3	34.9	33.3	33.3	33.5	32.6	33.2
3. Alveolar R.Q.	1.19	1.12	1.08	1.04	1.02	1.02	1.00	0.97	0.97	0.97	0.96	0.95	0.96	0.92	0.94
4. Ventilation l./min.	11.07	11.46	11.09	11.09	11.38	16.01	17.65	17.63	17.65	17.58	13.11	10.98	10.11	9.99	10.25
5. Breaths/min.	12	12	12	12	11	14	15	15	15	17	12	13	11	12	11
6. % Hb O_2 (Calc.)	85	83	83	82	82	78	78	78	77	78	80	80	80	80	79
7. % Hb O_2 (Oximeter)	81	80	78	78	78	74	73	73	72	71	74	74	76	76	75
8. Ml. O_2/min. (Calc.)	280	313	320	319	344	526	589	607	609	594	426	348	316	307	325
9. Heart Rate					95					107					92

Averages for Each Period

Series VIII.—6% CO_2	Normal	6% CO_2	Normal
1. Alveolar pO_2	47.9	48.3	46.4
2. Alveolar pCO_2	30.9	40.4	32.7
3. Alveolar R.Q.	1.12	0.82	1.16
4. Ventilation l./min.	12.99	14.84	12.70
5. Breaths/min.	11	13	11
6. Heart Rate	87	79	83
7. % HbO_2 (Calc.)	87	84	85
8. Oximeter	85	84	82

Series IX.—Work at Ground Level	Resting	Work	Recovery
1. Alveolar pO_2	101.4	97.2	102.4
2. Alveolar pCO_2	37.9	41.3	37.5
3. Alveolar R.Q.	0.84	0.83	0.85
4. Ventilation l./min.	8.86	13.76	9.22
5. Breaths/min.	12	15	13
6. Ml. O_2/min. (Calc.)	334	612	332
7. Heart Rate	84	92	87

Series X.—Work at 16,000 Feet	Resting	Work	Recovery
1. Alveolar pO_2	44.4	40.2	41.3
2. Alveolar pCO_2	33.5	35.2	33.2
3. Alveolar R.Q.	1.09	0.99	0.95
4. Ventilation l./min.	11.22	17.30	10.89
5. Breaths/min.	12	15	12
6. % Hb O_2 (Calc.)	83	78	80
7. % HbO_2 (Oximeter)	79	73	75
8. Ml. O_2/min. (Calc.)	309	585	344
9. Heart Rate	95	107	92

action of the initial anoxic stimulus which shortly is reduced in its net effectiveness because of the opposing inhibitory stimulus of acapnia. The pCO_2 tends to drop throughout the period of exposure to altitude, and as a consequence of this progressive acapnic state, the ventilation rate likewise gradually diminishes even

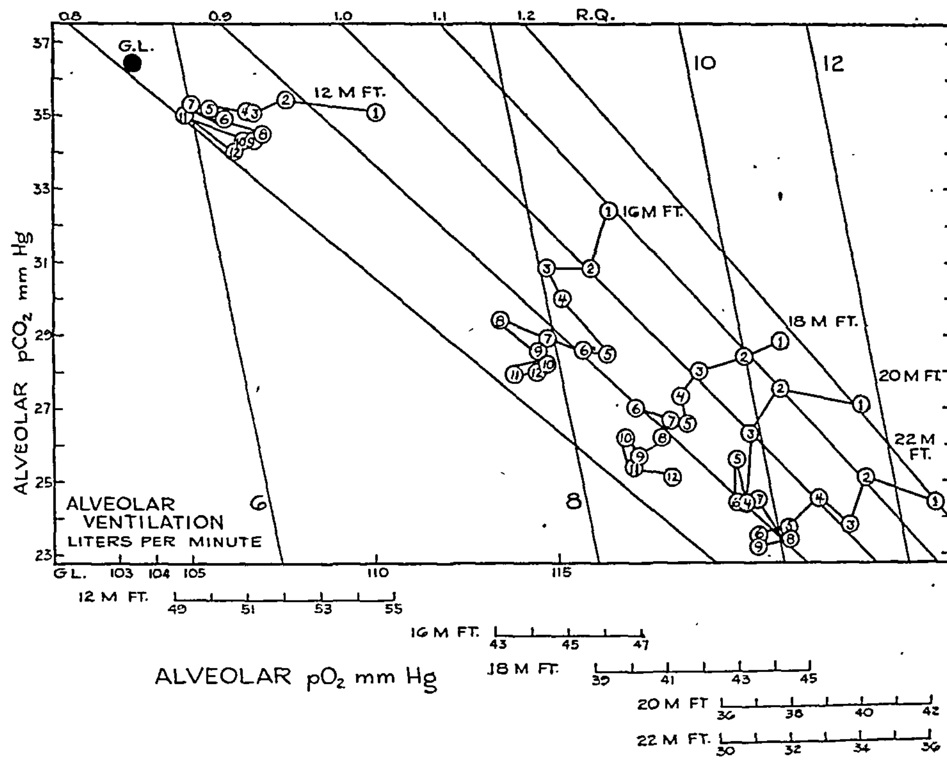

Fig. 1. The alveolar pathway during acute exposure to altitude.

Ordinates: Alveolar carbon dioxide tensions in millimeters of mercury.

Abscissae: Alveolar oxygen tensions in millimeters of mercury. Each of the multiple scales of abscissae applies to the family of points labelled with the corresponding altitude on the diagram.

Parallel diagonals: Isoventilation lines constructed by use of equation (19) from Fenn, Rahn and Otis, 1946.

Converging diagonals: Lines of constant R.Q. located by means of equation (9) from the same source.

The location of each member of the family of circles for a given altitude indicates the average composition of the alveolar air during a five-minute interval. The circles in each family are numbered consecutively, "1" indicating the first five minutes at altitude, "2", the second five minutes, etc. The solid circle indicates the average alveolar composition during the control periods at ground level, and hence is a sort of starting point from which changes may be followed as described in the text.

though the stimulus from anoxia is increasing. Comparing the different altitudes, one sees that the higher the altitude, the greater is the ventilation, the larger is the initial rise in the alveolar respiratory quotient, and the lower does the alveolar pCO_2 fall. The marked temporary gain in alveolar pO_2 by virtue of the initially high R.Q. is also readily apparent in this diagram. Lines indicating the

degree of oxygenation of the arterial blood could also be added but they would be different for different altitudes. By the end of the one hour exposure to altitude, the alveolar R.Q. has dropped nearly back to normal, indicating the approach to a new steady state. Although the 20,000 and 22,000 foot flights were not continued for this length of time, the trend of the points indicates that one hour would also suffice at these altitudes for attainment of an approximate steady state.

The alveolar pathway to this new steady state is not precisely linear, for the lines connecting the consecutive five-minute points form a zig-zag route which occasionally doubles back on itself. Some of these irregularities may be attributed to "hunting" on the part of the respiratory center for some minimum stimulus. Others may be due to extraneous sensory stimuli difficult to define or control. However devious the detailed path may be, there is no doubt as to the net direction. On teleological grounds this particular direction might be presumed to represent the points of optimum performance. Our evidence indicates, however, that in general the performance as measured by our tests is improved by ventilating somewhat faster than one actually does (Otis, Rahn, Epstein, and Fenn, 1946). Apparently the average subject tends to economize on respiratory effort somewhat to his own disadvantage. In exposures of greater duration the less well understood and more slowly acting processes of acclimatization must become of importance.

Only one of the eight subjects failed to complete the prescribed schedule of flights. It is of interest to note that this individual, who collapsed after ten minutes at 22,000 feet, consistently showed a smaller ventilation increase at altitude and consequently a higher alveolar pCO_2, and a lower oximeter reading than did the other subjects. Another subject, on the other hand, was somewhat inclined to nervous hyperventilation, but completed all flights without mishap.

The recovery at ground level following the flights was observed in a limited number of cases. The alveolar pathway upon recovery shows a reversal of the processes described above. Since the anoxic stimulus is no longer present, the acapnic inhibition of ventilation is free to exert itself until the CO_2 stores of the body are restocked. During this period of diminished ventilation, the alveolar R.Q. is lower than normal, and gradually rises to the resting value. The rate of return to normal pCO_2 values depends upon the previous anoxic hyperventilation at altitude. The higher and the longer the altitude exposure, the longer the recovery. The alveolar pathway is similar to that following hyperventilation as described by Rahn, Mohney et al., 1946. The R.Q. dips below 0.8 and slowly returns to normal with a consequent rise in pCO_2, pO_2 and ventilation rate as the CO_2 stores of the body are replenished. Thus acute exposure to altitude and recovery are represented on the CO_2-O_2 diagram by a closed circular or elliptical pathway bisected by the metabolic R.Q. line to which the body eventually returns both at altitude and during recovery from altitude. This is illustrated in figure 2 drawn from average values obtained on seven subjects after 30 minutes at 22,000 feet.

The value of the continuous sampling method in following the time course of

respiratory changes has been demonstrated above. As has been previously reported (Rahn, Otis, Mohney and Fenn, 1946) the continuous method gives values for the alveolar pCO_2 which are slightly lower and values for the alveolar pO_2 which are slightly higher than those given by the conventional Haldane-Priestley method. The additional data obtained in the present experiments bear this out. In table 5 the differences between the results of continuous analysis and analysis

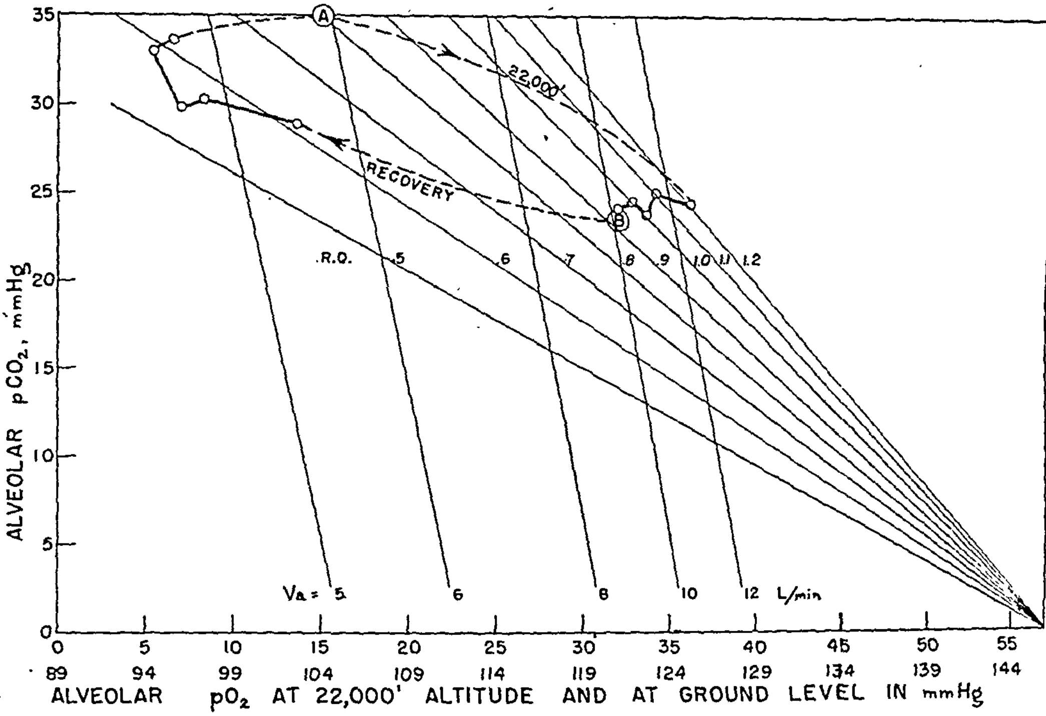

Fig. 2. Alveolar pathway before, during and after exposure to 22,000 foot altitude (7 subjects). Point A represents the average of 20 minutes at ground level. This is followed by a five minute ascent to 22,000 feet during which no record was taken. The small circles are consecutive five minute averages after arrival at 22,000 feet. B represents the end of thirty minute exposure and beginning of descent. No record was taken during five minute descent. The small circles during the recovery cycle are again averages of consecutive five minute intervals during the first twenty-five minutes after arrival at ground level. (Alveolar Ventilations are calculated but agree well with observed Total Ventilation Values.)

of end-expiratory Haldane-Priestley samples are compared. Of particular interest is the fact that the discrepancy between the two methods becomes less as the altitude increases. This may be due to the increasing ventilation rate. Another point of interest in comparing the continuous samples with the forced expirations is that the per cent saturation of the arterial blood estimated from the alveolar pCO_2 and pO_2 with the nomogram of L. J. Henderson (1928) agrees more closely with oximeter readings when the values obtained from forced samples are used (table 6). The values from the continuous method consistently give percentage saturations which are higher than the oximeter readings.

The reproducibility of measurements by continuous sampling is indicated by a comparison of the data of series III and III A, both of which represent normal respiratory behavior of our subjects at 18,000 feet. The results of the two series appear not to be significantly different.

TABLE 5

The discrepancy between the continuous and the conventional methods of alveolar air analysis at various altitudes

(1) ALTITUDE	(2) n	(3) MEAN pCO_2 DIFFERENCE	(4) S.D.M.	(5) MEAN pO_2 DIFFERENCE	(6) S.D.M.
Ground Level	112	2.00	0.18	4.06	0.46
12,000	98	2.20	0.17	3.71	0.27
16,000	41	2.38	0.22	3.80	0.53
18,000	121	1.61	0.14	2.95	0.21
20,000	32	1.38	0.19	2.76	0.56
22,000	21	0.83	0.25	1.80	0.31

The meaning of the columns is as follows:

(1) Simulated altitude in feet. (2) The number of comparisons. (3) The mean difference (mm. Hg) in values for alveolar pCO_2 obtained by the two methods. (The continuous method always gives the lower values.) (4) The standard deviation of (3). (5) The mean difference (mm. Hg) in values for alveolar pO_2 obtained by the two methods. (The continuous method always gives the higher values.) (6) The standard deviation of (5).

TABLE 6

Comparison of values for percentage saturation of arterial blood estimated from the two methods of sampling alveolar air with those given by the oximeter

ALTITUDE FEET	CONTINUOUS SAMPLER			FORCED EXPIRATION			Oximeter Hbo_2
	pCO_2	pO_2	Calc. Hbo_2	pCO_2	pO_2	Calc. HbO_2	
	mm. Hg	*mm. Hg*	*per cent*	*mm. Hg*	*mm. Hg*	*per cent*	*per cent*
12,000	34.8	51.0	87	37.0	47.3	85	86
16,000	29.3	44.7	84	33.7	40.9	79	80
18,000	26.8	41.3	82	28.4	38.3	79	77
20,000	25.1	37.3	79	26.5	34.5	75	75
22,000	24.3	33.4	74	25.1	31.6	69	67

2. *The Effect of Glucose on the Alveolar Pathway.* The flights of series VI and VII were performed to test the effects of glucose ingestion on the alveolar pathway at 12,000 and at 18,000 feet. Previous work on the effect of carbohydrate ingestion on respiratory behavior has been reviewed by Ivy, Friedman and Grodins, 1944. Beneficial effects of carbohydrate diets in high altitude flights were reported by these authors as well as by King et al. (1945a), (1945b), Barach et al. (1945) and others.

If the figures in series VI and VII are plotted on a chart like figure 1 and the resulting graphs are compared with control values (series I and III or III A respectively) it is evident that at 12,000 feet glucose had a slight effect. At

18,000 feet, however, the R.Q. and pCO_2 were both definitely elevated. This confirms the theoretical expectations although the pO_2 was, if anything, somewhat diminished rather than increased by the glucose. If the sugar had been taken slightly earlier the result might have been even more definite. The result is attributable to an increased metabolic R.Q. as well as increased CO_2 liberated from bicarbonate by increased blood lactate.

3. *Work at Ground Level and Altitude* (series IX and X and table 4). The work output per minute was calculated at 49.4 kgm. This is the equivalent of 23.9 cc. of oxygen burning food with a respiratory quotient of 0.831. The calculated extra oxygen consumption required for this work was 277 cc./min. at ground level and at 16,000 feet altitude giving a net efficiency of 8.6 per cent. At both pressures the alveolar oxygen pressure dropped 4 mm. Hg during the work period

TABLE 7

Comparison of observed and calculated ventilation ratios in muscular work

GROUND LEVEL	ALVEOLAR VENTILATION	VENTILATION RATIO	
		OBSERVED	CALCULATED
	L/min.		
Rest	6.34	1.0	1.0
Work	10.61	1.67	2.31
16,000 FEET			
Rest	8.70	1.37	1.23
Work	14.15	2.23	2.99

The V.R. was calculated by Gray's equation:

$$V.R. = 0.4\,pCO_2 - 14.21 + \frac{105}{10^{.038\,pO_2}}$$

The constant 14.21 was used instead of 15 to make the ground level resting value = 1.0 with a pCO_2 of 37.9. Data for pO_2 and pCO_2 are in summary in table 4.

while the carbon dioxide increased. This change is sufficient to account for the decrease in oxygen saturation of the blood. The calculated oxyhemoglobin saturation parallels the observed ear-oximeter readings.

The values of the alveolar ventilation are calculated in table 7 for rest and work at ground level and 16,000 feet using the summary data in table 4 and a dead space of 210 cc. It is evident that the same work requires an increment of 5.45 liters/min. at altitude and only 4.25 at ground level. This fact was also observed by Gray (1945). All these ventilation rates are reasonably well explained by the use of Gray's equation as given in table 7. The observed and calculated ventilation ratios listed in table 7 increase in the same order and the numerical values are not grossly different. This shows that the recorded changes in alveolar pO_2 and pCO_2 are consistent with the measured ventilation rates.

4. *Heart Rate Changes During Exposure to Simulated Altitudes.* Heart rates were measured in the above flights to obtain some measure of the circulatory stress involved. With the exception of the 12,000 foot value, which seems to be

out of line with the rest of the data, the rate increases progressively with altitude. At the highest altitude (22,000 ft.) it is 127 per cent of the ground level rate.

The rates obtained during the flights following glucose ingestion are considerably higher than the corresponding values for the normal series (except at 12,000 ft.). An increased heart rate as a result of the glucose would not be surprising, since Boas and Goldschmidt (1932) found that the heart rate tends to be higher after a meal than before. Grollman (1929) found that the cardiac output is likewise higher after the ingestion of food.

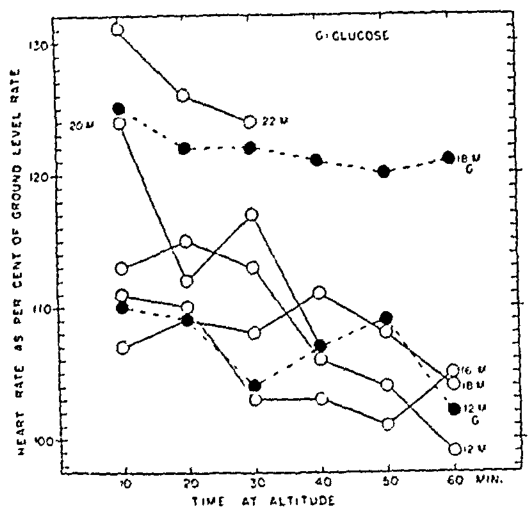

Fig. 3

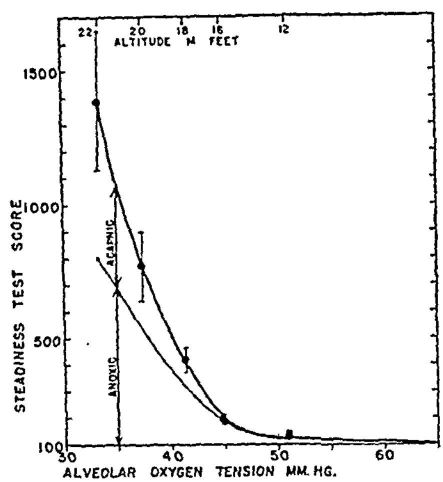

Fig. 4

Fig. 3. Heart rate as affected by time at altitude.
Ordinates: Heart rate as percentage of ground level rate.
Abscissae: Time at altitude in minutes.
Open circles: Data from series I–V (normal series).
Closed circles: Data from series VI and VII (glucose series).

Fig. 4. Steadiness test score as related to alveolar oxygen tension and altitude.
Ordinates: Steadiness test score as percentage of ground level score (mean of eight subjects).
Abscissae: Scale at bottom: Alveolar pO_2 in millimeters of Hg.
Scale at top: Altitude in thousands of feet.
The vertical line through each point indicates the standard deviation of the mean.
The lower of the two curves represents the score expected from anoxia alone, and was constructed by the method referred to in the text.

The change in heart rate with time at altitude is of interest. Figure 3 shows that the rate is, in most cases, highest during the first 10 minutes at altitude. During the time of exposure it tends to drop progressively. In the 12,000 foot series it had returned to the normal ground level rate by the end of the hour. This tendency of the heart rate to return toward the normal with time has been observed previously by McFarland (1938). It may represent a part of the adaptation process of the body to low oxygen tensions. It would be of interest to know whether it is accompanied by an increase in the stroke volume or in the coefficient of utilization.

5. *Performance on the Hand Steadiness Test during Exposure to Altitudes.* Results of the steadiness tests which were administered in flight series I–V are summarized in figure 4. The mean of all scores at each altitude is plotted against the mean alveolar oxygen tension. A barely significant change in score occurs at 12,000 feet. At the higher altitudes the score grows progressively worse. The standard deviation of the mean score also increases with altitude.

An estimate has been made as to how much of the deterioration at each altitude is due to anoxia and how much to acapnia. This was done by the method described in a previous report for the construction of a pure anoxia curve (Otis, Rahn, Epstein, and Fenn, 1946). The acapnic component was estimated by the use of figure 6 of the report just cited. The result of this analysis is shown on figure 4, and indicates that a major part of the deterioration is due to anoxia. At the higher altitudes the acapnic fraction becomes significant, however.

The ingestion of glucose appeared to have no significant effect on steadiness test performance. The scores for the glucose flights at 12,000 and 18,000 feet are almost identical with the scores obtained in the normal flights at these altitudes.

6. *Inspired Carbon Dioxide at 16,000 Feet.* The effect of addition of 6 per cent carbon dioxide to the inspired air at altitude is well illustrated by figure 5. On this diagram is plotted the time course of change in alveolar composition during the experiments of series VIII. (For data see table 3.)

During the initial ten minute period of normal air breathing at 16,000 feet, the alveolar R.Q. is always above 1.0 but steadily drops as does the alveolar pO_2. The ventilation also gradually decreases. With the introduction of 6 per cent CO_2 in air the alveolar pCO_2 rises by about 10 mm., and the R.Q. drops because CO_2 is being retained by the body. This rise in pCO_2 is accompanied by an increase in ventilation which, in turn, results in a gain of about 4 mm. oxygen tension in spite of the high CO_2. For a theoretical discussion of this phenomenon see Fenn et al. (1946) or Gray (1944). By the end of 10 minutes of CO_2 breathing the R.Q. has risen to a normal level of 0.85 indicating the approach of a new steady state.

Attention should be called to the fact that upon addition of 6 per cent CO_2 to air the inspired O_2 tension at 16,000 feet is reduced by 5 mm. Hg. If the inspired O_2 per cent had been kept at 20.9 per cent the addition of 6 per cent CO_2 would have resulted in an alveolar oxygen gain of 5 mm. Hg above that observed and would have resulted in an increased oxyhemoglobin saturation of 3 per cent. However, from a practical standpoint, the substitution of the same volume of O_2 for CO_2 would increase the inspired O_2 to 25.6 per cent representing a gain of 17 mm. Hg in the alveolar air without the concomitant hyperventilation.

During the recovery period on air the reverse changes occur and at the end of 10 minutes the alveolar composition is about the same as immediately before the introduction of CO_2. Had the whole 35 minutes been spent on air, the alveolar pCO_2 and pO_2 would probably have continued to drop. The alveolar composition at the end of 35 minutes in the experiments of series II (normal flights to 16,000 ft.) is indicated on the chart by a solid circle.

The lines of equal ventilation, Va, plotted in figure 3 represent only the alveolar ventilation. According to the chart the addition of 6 per cent CO_2 increased the ventilation from about 10 to about 13 liters per minute. In theory these

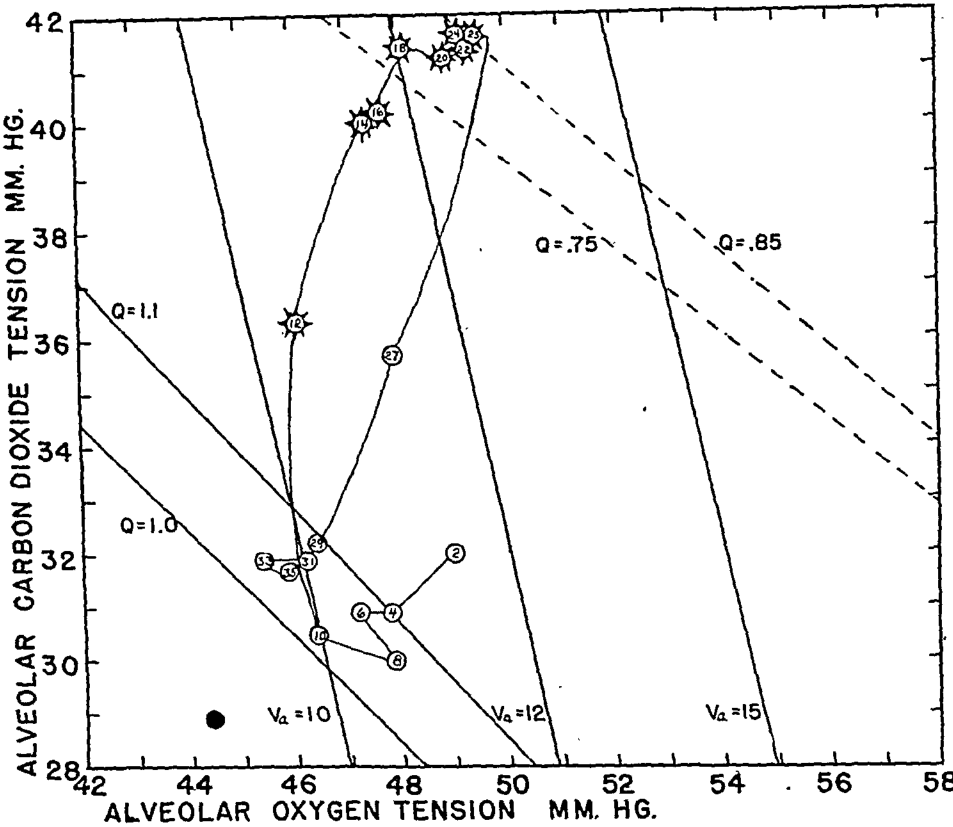

Fig. 5. The effect of 6 per cent carbon dioxide in the inspired air on the alveolar pathway at 16,000 feet.

Ordinates: Alveolar carbon dioxide tensions in millimeters of mercury.

Abscissae: Alveolar oxygen tensions in millimeters of mercury.

Parallel diagonals: Isoventilation lines for alveolar ventilations of 10, 12, and 15 liters per minute constructed from equation (19) of Fenn, Rahn and Otis (1946) using a value of 346 cc./min. for the oxygen consumption.

Converging diagonals: Lines of constant alveolar R.Q. located by use of equation (6) from the same source.

The solid R.Q. lines apply to air breathing, the broken lines to breathing 6.0 per cent CO_2.

The location of each circle indicates the composition of the alveolar air at the elapsed time in minutes indicated by the number in the circle. Plain circles refer to air breathing, and starred circles to CO_2 breathing. The solid circle indicates the composition of alveolar air at the end of 35 minutes in the experiments of series III.

values apply to any subject at an altitude of 16,000 feet who has an oxygen consumption of 346 cc. per min. The approximate accuracy of these calculated values is indicated by the observed average values in table 4 which show that the total ventilation increased from 12.14 to 15.37 l./min. as a result of the added CO_2. With an assumed dead space of 210 cc. this means an increase of alveolar

ventilation from 10.04 to 12.64 l./min. (cf. 10 and 13). With a slightly greater increase in ventilation the arterial saturation in the 6 per cent CO_2 period would have been greater than in the control but in this case there was on the average no essential change. The large increase in pCO_2 however enabled the subject to attain a higher alveolar pO_2 than would have been possible had he remained longer at the same altitude, breathing air.

SUMMARY

1. The alveolar air composition, ventilation volume, breathing rate and oxyhemoglobin saturation were recorded continuously and heart rate and hand steadiness intermittently on 8 subjects in 88 man flights (a) to altitudes of 12, 16, 18, 20, and 22 thousand feet; (b) to 12 and 18 thousand feet with and without previous administration of glucose; (c) to 16 thousand feet with muscular work, and (d) to 16 thousand feet with the addition to the inspired air of 6 per cent CO_2.

2. The respiratory data illustrate the increase in R.Q. and gradual return to normal during the hour at altitude as well as the subnormal R.Q. and return to normal during recovery. The time course of these changes is illustrated on a pO_2-pCO_2 chart.

3. The increased ventilation at altitude was accomplished by increase in tidal volume only, except at the 22,000 foot altitude. Ventilation was maximum during the first five minutes at altitude.

4. Heart rates showed an immediate increase on exposure to altitude, and dropped toward normal with continued exposure.

5. Hand steadiness showed a barely significant change at 12,000 feet, and grew progressively worse with increasing altitude.

6. The most conspicuous effects of previous glucose ingestion were an increased R.Q. and an augmented heart rate.

7. A given amount of muscular work increased the ventilation more at 16,000 feet than at ground level, but lowered the alveolar pO_2 by the same decrement under both conditions.

8. Addition of 6 per cent CO_2 to the inspired air at 16,000 feet increased the alveolar pCO_2 10 mm. and the pO_2 4 mm.

9. Alveolar samples obtained by the continuous method show a higher pO_2 and a lower pCO_2 than those obtained by forced expirations, but the discrepancy becomes less as the altitude increases.

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RETENTION OF NORMAL INSULIN TOLERANCE AND ADRENAL CORTEX AFTER EXTIRPATION OF THE HYPOPHYSIAL STALK IN THE DOG[1]

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It has recently been found that the magnitude of both the decrease in tloerance for insulin and the atrophy of the adrenal cortex increased, when, in addition to removal of the pars anterior and the posterior lobe, the tissue of the hypophysial stalk was progressively encroached upon (1). It was also reasonably certain that these functions were not materially affected by total removal of the pars anterior tissue, providing sufficient stalk tissue was left undisturbed. From these observations, it was concluded that hypophysial stalk tissue, presumably the pars tuberalis, possesses the ability to elaborate the autacoids which normally maintain these functions.

The question was immediately raised as to whether the elaboration of these autacoids was specifically limited to stalk tissue, or whether such elaborating powers were common to both the pars tuberalis and the pars anterior.

In the event the former situation obtains, removal of the major portion of the stalk tissue, in spite of a sparing of distal pars anterior tissue, should precipitate the same degree of deficits which is encountered following near-total hypophysectomy (1). If the latter situation obtains, this procedure should be accomplished without precipitating noteworthy deficits.

This report describes the results obtained in a small series of dogs in which such a selective removal of the hypophysial stalk tissue was accomplished, and in which the results are unequivocal.

METHODS. *Operative procedures.* After the right lateral aspects of the hypophysis, the stalk, and the hypothalamus were exposed by the subtemporal approach, as previously described (1), the stalk tissue was removed in one of the following two ways.

In the first part of the series the stalk was cut distally with one snip of the scissors so that a good rim of pars anterior tissue remained attached to the stalk tissue (see line 2, fig. 1A). A small metal disc was then placed against the cut surface of the isolated hypophysial tissue to protect this tissue from the heat of the cauterization probe. The stalk tissue left dangling from the hypothalamus was then cauterized.

This method was soon discarded because of 1, the uncertainty of devitalizing all the stalk tissue, and 2, the frequent encroachment upon the tuberal portion of the hypothalamus. Instead, after the stalk is cut with one snip of the scissors as close to the hypothalamus as possible (see line 1, fig. 1A), a second cut

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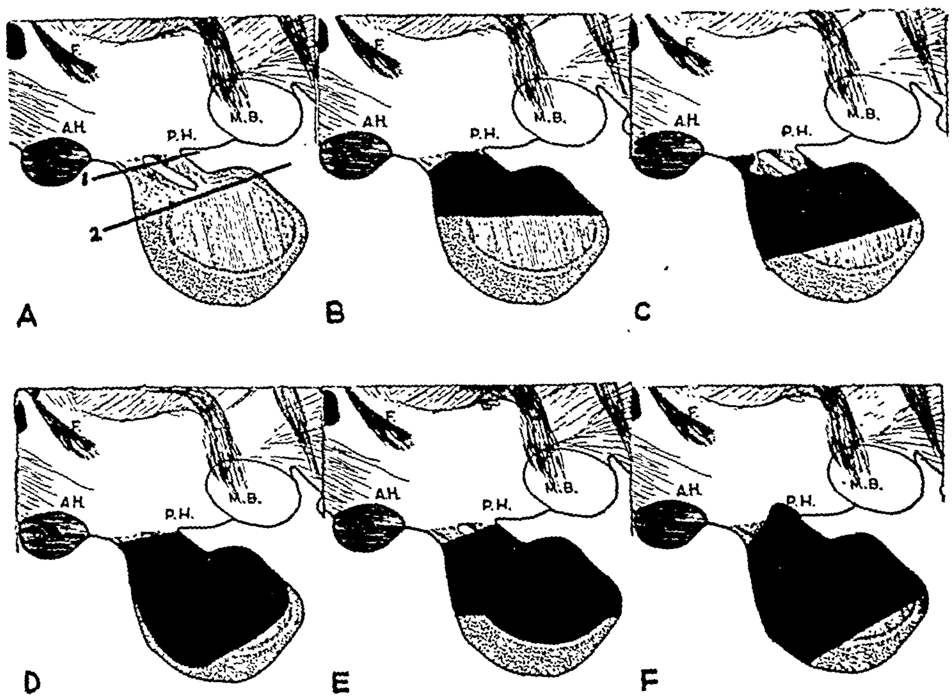

Fig. 1. Schemata illustrating the surgical procedure attempted and the surgical results attained in several of the dogs.

In A is shown the schema of a sagittal section through the hypophysis and hypothalamus of the dog, upon which lines 1 and 2 are superimposed to indicate the relative position of the two scissor cuts made through the stalk. The tissue between the two lines was removed as a surgical specimen or destroyed by cautery in the stalk removal procedure. The exact placing of the scissor cuts, of course, varied, as did the disappearance of tissue bordering the scissor cuts due to disturbances in blood supply.

In B, C, D, E, and F, the blacked-out area is the estimated amount of and location of the hypophysial tissue found to be absent, as determined from inspection of representative sections taken from the series on these dogs.

B. Location and extent of the lesion in dog 1. Note that only a small proximal fragment of tuberalis and infundibulum tissue remained attached to the hypothalamus. See figures 2A and 3A.

C. Location and extent of the lesion in dog 2. Note that only a very small fragment (posteriorly) of the proximal tuberalis remained, but that rather a large fragment of the proximal infundibulum tissue remained undisturbed. In this dog, the edges of the two scissor cuts are readily seen on the section, but it is to be noted that considerable of the tuberalis tissue disappeared proximal to section 1. See figures 2B and 3B.

D. Location and extent of the lesion in dog 3. Note that only a very small remnant of the proximal tuberalis and infundibulum remained attached to the hypothalamus. Also, that there was considerable disappearance of the hypophysial tissue distal to scissors cut no. 2. See figures 2C and 3C.

E. Location and extent of the lesion in dog 4. Note that some tuberalis and infundibulum tissue remained attached to the hypothalamus. Also that the entire posterior lobe was absent except for a few small islands of intermedia tissue. See figures 2D and 3D.

F. Location and extent of the lesion in dog 5. Note that there was a small fragment of the tuberalis and infundibulum left attached to the hypothalamus anteriorly, and that there was a just perceptible gross infringement upon the hypothalamic tissue posteriorly. Also that only the extreme distal portion of the pars anterior tissue remained viable. See figures 2E and 3E.

is made through the proximal portion of the pars anterior (see line 2, fig. 1A). The tissue freed by the two cuts is picked up as a surgical specimen and stored in formalin for reference. This procedure prevents encroachment upon the hypothalamus, as well as leaving attached to the hypothalamus more than a remnant of the proximal stalk.

Tissues. The routine handling and staining of tissues has been previously described (1).

RESULTS. *Accomplishment of operative objective.* The selective removal of the hypophysial stalk tissue was attempted in 15 dogs. The hypophysial stalk tissue, except for small fragments of the proximal tuberalis and infundibulum which remained attached to the hypothalamus, was successfully removed

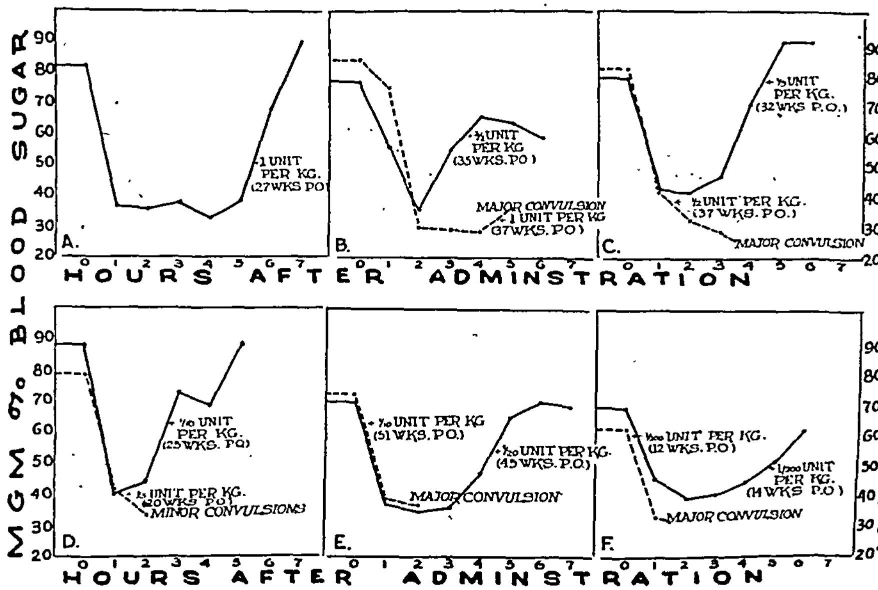

Fig. 2. Insulin tolerance curves for dogs 1 to 6. Compare with figures 1 and 3.

in 11 of the 15 animals. Three of these were complicated by a slight infringement upon the hypothalamus. In the remaining 8 dogs the hypothalamus remained undisturbed; accordingly, the operative objective was accomplished in 8 animals. In all of these a sizable portion of the isolated pars anterior tissue remained viable, but the estimated percentage of the total amount varied considerably, as is shown by the schemata in figure 1.

Tolerance for insulin. Five of the eight dogs successfully tolerated ½ or 1 unit of insulin per kilogram of body weight, and accordingly they showed no appreciable change from the normal in this function. The three remaining dogs exhibited a definite decrease in insulin tolerance (5 to 20 times), but this was far from maximal (80 to 200 times). The results of the assay of insulin tolerance in five of these dogs are shown in figure 2. The operative results on these same dogs are shown in figure 1.

Adrenal cortex size. At autopsy the adrenal glands of all eight dogs were

within what might be considered to be the normal range in over-all size, shape, color and thickness of the cortex. Although partial atrophy was suspected in some cases, certainly in no instance was there anything akin to the degree of

Fig. 3. Photographs of the left adrenals of dogs 1 to 7. Compare the over-all size and the width of the cortex of each gland with the other glands, but particularly with the one in G, where cortical atrophy was maximal. In making these comparisons, keep in mind the weights of the individual animals.

A. Dog 1, at the time of operation, was an adult female weighing 10 kgm. It was maintained 4 months after the stalk removal operation, at which time it weighed 13 kgm. See figures 1B and 2A.

B. Dog 2, at the time of operation, was an adult female weighing 10.5 kgm. It was maintained for 25 months subsequent to the stalk removal procedure, at which time it weighed 10 kgm. See figures 1C and 2B.

C. Dog 3, at the time of operation, was what was judged to be a three-fourths grown female puppy, but exact age was unknown, weighing 4 kgm. It was maintained for 14 months after the stalk removal procedure. At the time of pancreatectomy performed 56 weeks after the first operation, the dog weighed 6 kgm., and was sexually mature. See figures 1D and 2C.

D. Dog 4, at the time of operation, was a sexually immature "senior pup", weighing 7 kgm. It was maintained for 10 months subsequent to the stalk removal procedure, at which time the animal was still sexually immature and weighed 7.5 kgm. See figures 1E and 2D.

E. Dog 5, at the time of operation, was a sexually immature female "senior pup" weighing 5.5 kgm. It was maintained for one year after the stalk removal procedure, at which time it was still sexually immature and weighed 7.5 kgm. See figures 1F and 2E.

F. Dog 6. In this dog an ordinary hypophysectomy was attempted and one intermediate between a near-total and an ordinary one was attained. At the time of operation, it was an adult female weighing 8.5 kgm. It was maintained for 49 postoperative weeks, at which time it weighed 10 kgm. See figure 2F.

G. Dog 7. The operation attempted and attained in this dog was an extreme near-total hypophysectomy. At operation it was an adult female weighing 9.5 kgm. Subsequent to operation, anterior lobe extract (Parke-Davis) and adrenal cortex extract (Upjohn) were administered daily for short intervals on several occasions. The dog died in a crisis 8 months after operation, at which time it weighed 8 kgm.

atrophy which is routinely encountered in the chronic near-total hypophysectomized preparation (1). These features are apparent from the photographs shown in figure 3.

Adrenal cortical function. In no animal was there any outward manifestation of adrenal hypofunction. If partial cortical atrophy was present, cortical

function was not reduced in any instance to anywhere near a critical level. This was evidenced by the fact that these animals 1, withstood the relatively severe surgical stress of pancreatectomy without untoward effects, and 2, they tolerated an environmental temperature of 5°C. for an eight-hour period without showing any deviation from the normal in rectal temperature. Such

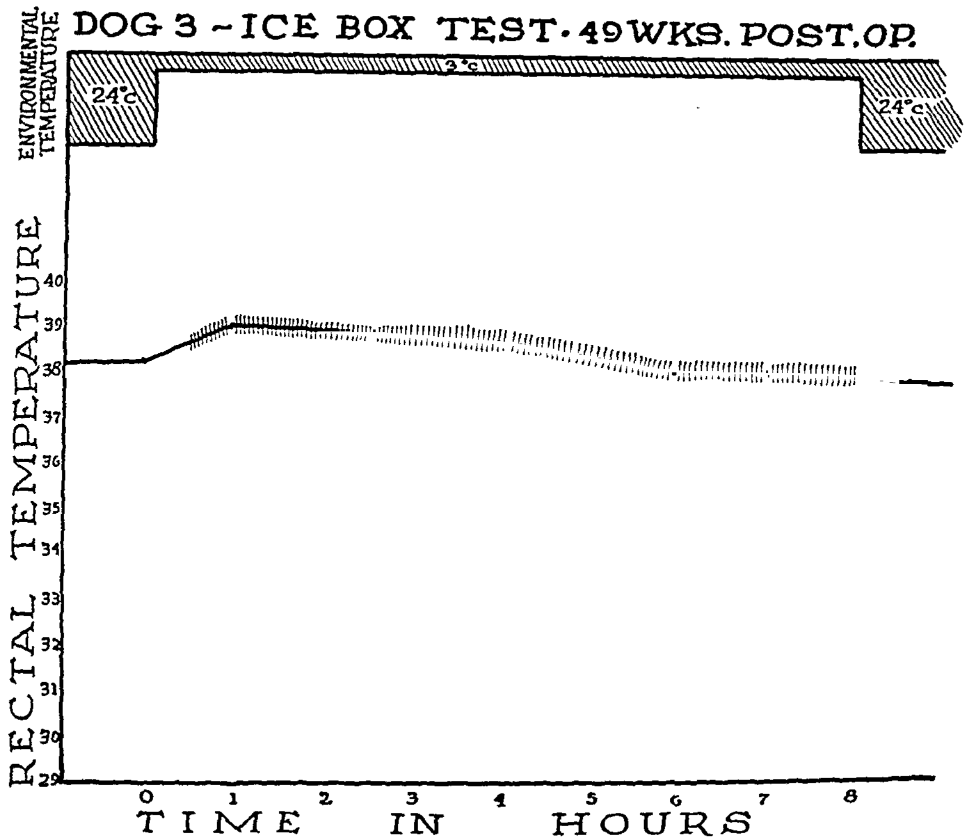

Fig. 4. Icebox test curve for dog 3. The test was run 49 weeks after operation.

a test for dog 3 is shown in figure 4. Both the environmental temperature tests and the pancreatectomies[2] were carried out several months after the

[2] The functional effects of pancreatectomy in this series have been interesting in two respects. First, of two animals which had lesions that appeared to be homologous, one exhibited typical diabetes mellitus (dog 1; see figs. 1B, 2A, 3A), while the other (not illustrated) exhibited a near-maximal Houssay effect. The other animals of the series exhibited diabetic levels between these two extremes, but a tendency toward the Houssay effect predominated. The animal exhibiting the near-maximal Houssay effect displayed no noteworthy deviation from the normal in any other function, save that of having a hyperactive sex drive (male).

The other feature of considerable interest, which has been encountered in this as well as in related series, is the apparent selective dissociation of post-pancreatectomy disturbances in carbohydrate metabolism from those of fat metabolism. Thus we have a few isolated protocols where the dogs developed no post-pancreatectomy hyperglycemia or glycosuria, but severe acidosis developed progressively and rapidly with death occurring in 6 to 10 days;

hypophysial surgical procedure, thus allowing more than adequate time for possible deficits to become fully developed and stabilized.

Discussion and conclusions. *Gross delimination of elaborating tissue.* It is evident that elaboration of the hypophysial principles, which maintain the adrenal cortex and its functions and the ability of an animal to tolerate normally a dose of insulin, is not restricted to the stalk tissue. Conversely, it is equally evident that the distal tissue of the pars anterior possesses these elaborating powers. Accordingly, the possibility that we might be dealing with an overlapping of tuberalis elements into the proximal portion of the pars anterior is ruled out. It is also evident that a relatively small amount of pars anterior tissue is capable of maintaining these functions within what is considered the normal range.

The question of the elaborating elements. The question naturally arises, "What are the cellular elements which are responsible for the elaboration of the endocrine factors under consideration?" As yet our histological studies have yielded nothing approaching a definitive answer. However, it might not be amiss to comment that in nondifferential staining procedures, the chromophobic population of the pars anterior does not differ radically from the predominant cellular population of the pars tuberalis. Save for the delimiting line made by the prominence of the acidophiles in the pars anterior and their abrupt sparsity in the pars tuberalis, even following differential staining procedures, it still remains a problem to ascertain histologically the exact line of demarcation between these two subdivisions of the adenohypophysis in the dog. Also, on the basis of a few isolated but distinctive protocols taken from our series, it appears that sex deficits and dwarfing can be precipitated by the stalk removal procedure without noteworthy deficits in carbohydrate and adrenal functions. Histologically the isolated remnant of the pars anterior exhibited marked variations in the basophilic and acidophilic elements, but no noticeable deviation from the normal in the chromophobes. On the basis of these observations, we, momentarily at least, are thinking in terms of the responsible elaborating elements as being aligned with the chromophobic cellular population of the adenohypophysis rather than with the acidophilic or basophilic elements. We are aware that currently the chromophobe cells are not allocated a secretory function (2), but we see no convincing reason for discounting such a probability. In fact, we are also inclined to think problematically in terms of there being functionally several types of chromophobic cells even though histologically they are indistinguishable. How else can one explain the selective dissociation of the various hypophysial deficits—resulting presumably from surgical sequelae—following graded hypophysectomy procedures?

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and in other instances there occurred a typical *maximal* hyperglycemia and glycosuria, but no corresponding acidosis.

These findings must, of course, be checked carefully in a much enlarged series of animals, but they are sufficiently striking and problematically suggestive to warrant mention in this connection and in this manner.

Correlation between decrease in insulin tolerance and atrophy of adrenal cortex. There has been a good deal of speculation pertaining to the relative parts played by the direct effect of hypophysial absence and the indirect effects of adrenal cortical atrophy in the over-all decrease in insulin tolerance following hypophysectomy. In this connection, it is pertinent to call special attention to the profound decrease (200 times) in insulin tolerance which obtained in dog 6 (fig. 2F) in the presence of only a moderate atrophy of the adrenal cortex (fig. 3F). Here is an instance where there was essentially a complete dissociation of these two hypophysial functions.

SUMMARY

The tissue of the hypophysial stalk which is removed in executing a near-total hypophysectomy was removed with a sparing of a sizable amount of the distal pars anterior tissue in 8 dogs (stalk removal procedure).

Most of the dogs subsequently showed no noteworthy deviation from the normal in 1, the size of or the function of the adrenal cortex, and 2, the ability to tolerate one-half to one unit of insulin per kilogram of body weight administered subcutaneously. Some atrophy in the adrenal cortex was suspected and a decrease in insulin tolerance was definite in a few of the animals, but in no instance did these deficits approach in magnitude those which are routinely encountered in the near-total hypophysectomized dog.

On the basis of these results and those previously published (1), it is concluded that the hypophysial control over the adrenal cortex and the over-all carbohydrate reserves (as reflected by insulin tolerance) are mediated by elements located in the cellular population of both the pars tuberalis and the pars anterior.

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QUANTITATIVE STUDIES OF CHRONIC FACILITATION IN HUMAN MOTONEURON POOLS[1]

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From the Still Memorial Research Trust, Kirksville, Missouri

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Previous studies (2, 3, 4) have indicated the existence, in man, of pools of spinal extensor motoneurons which are in a state of enduring excitation, as reflected in low reflex thresholds. These were reproducible over periods of months. Persistent differences in threshold were found from subject to subject, among segments of the same subject, and between sides of the same segment.

This paper reports the following: *a*, the correlation of reflex threshold with other segmental features, and *b*, the intersegmental spread of excitation. Previous observations of threshold differences were confirmed with improved technics.

METHODS. All the subjects were young men apparently in good health. Each electrode, a bare 1 inch 25 gauge hypodermic needle, was inserted perpendicularly to the skin, through a procaine wheal 3 cm. to the left of the tips of the spinous processes T_4, T_6, T_8, and T_{10}, into the underlying erector spinae mass. Each muscle electrode was coupled with another 25 gauge needle which was inserted intradermally through the wheal. This type of electrode pairing permitted a higher degree of localization and sensitivity than the previous practice (2) of pairing 2 electrodes in muscle, 1 segment apart. Action potentials were recorded as previously described (2).

The reflex threshold of a given segment was obtained by determining the lowest pressure, applied over the spinous process of that segment, which elicited spike potentials from the spinal extensor at the same level.[2] Measured pressure stimuli were applied over the spinous process by means of the pressure meter previously described (2). To simplify tabulation, responses to pressure stimuli were grouped and designated as follows: 1–2 kgm. Low (L); 3–5 kgm.

[1] These studies were supported by grants from the Research Fund of the American Osteopathic Association.

[2] It is not intended to imply that the sensory fibers at the spinous process and motoneurons of the muscle at that level both emerge from the same segment of the cord, since such an anatomic relationship has not been demonstrated. It is probable, however, that a given spinous process bears a closer neural relationship to neighboring muscle segments than remote ones and that there is more or less parallel segmental "spacing" in the cord.

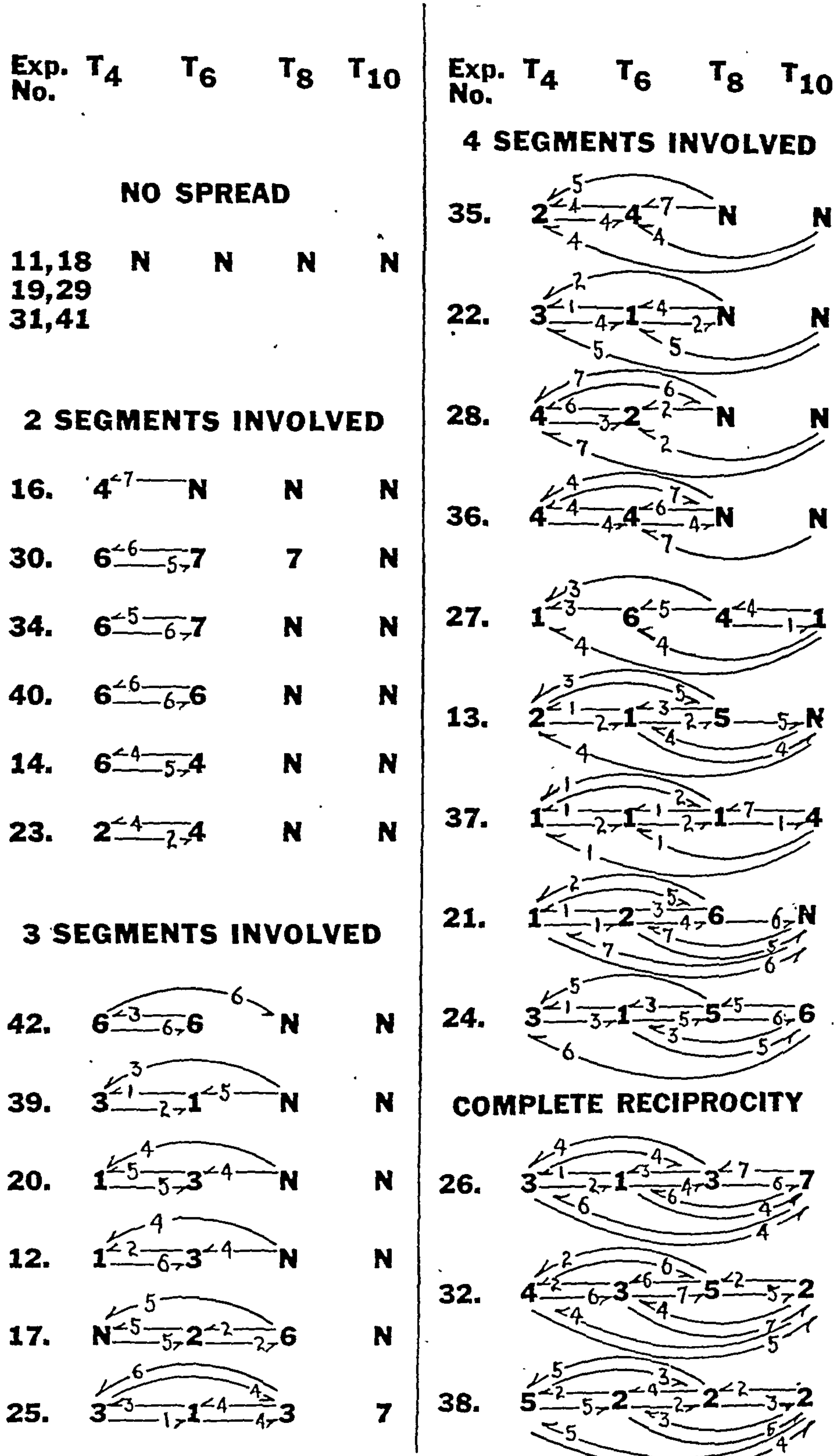
Exp. No. T4 T6 T8 T10
NO SPREAD
11,18 19,29 31,41 N N N N
2 SEGMENTS INVOLVED
16. 30. 34. 40. 14. 23.
3 SEGMENTS INVOLVED
42. 39. 20. 12. 17. 25.
Exp. No. T4 T6 T8 T10
4 SEGMENTS INVOLVED
35. 22. 28. 36. 27. 13. 37. 21. 24.
COMPLETE RECIPROCITY
26. 32. 38.

Fig. 1

Medium (M); 6–7 kgm. High (H); and no activity at the top limit (7 kgm.) of the stimulator, None (N). In figure 1, however, the actual thresholds are given.

In addition to establishing the local threshold for each of the four segments, the pressure required at each of the four spinous processes to elicit reflex activity from each of the other three recording sites was determined. In every experiment, therefore, the thresholds of 16 related reflex pathways were established. These furnished the data also for determining the extent and facility of spread of excitation among the segments studied.

RESULTS. *Part I. Threshold Differences; Segment to Segment and Subject to Subject.* Figure 1 (arrows are to be disregarded until a later section) shows the differences in (local) threshold that may exist among the 4 selected segments of a given subject, and among corresponding segments in different subjects. Thresholds of 1–5 kgm., called L and M in other charts, occur most frequently at T_4 and T_6 and thresholds of 6 kgm. and more (H and N) at T_8 and T_{10}.

Constancy of Reflex Thresholds. Thresholds remained essentially constant over periods of months. The largest difference between duplicate determinations was equivalent to one threshold group and even this difference was not common.

Threshold Differences Between Right and Left Sides. In a previous study on 30 subjects (2) it was shown that the mean threshold-segment curves of the right and left sides did not coincide, considerable divergence occurring at some levels. Two experiments, with the improved electrode technic introduced in this study, confirmed the existence of threshold differences between the right and left side of the same segment.

In one experiment, the threshold of T_8 was N (over 7 kgm.) for the right musculature and, at the same time, 2 kgm. for the left. In the other, the right side of T_6 responded to 1 kgm. of pressure over the spinous process, while the left side failed to respond when the top limit of the stimulator (7 kgm.) was applied over the same spinous process.

Location of the Sensory Element. When the periosteum and other tissues closely investing the tips of spinous processes of low threshold segments were procainized, the thresholds were considerably elevated, often beyond the limit of the pressure meter. In contrast, procainization limited to the skin over-

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Fig. 1. All 30 experiments are diagrammed and arranged according to pattern. The bold type numeral under each segment represents the "self-threshold" (discussed in part I) in kilogram for that segment, e.g., the pressure on the spinous process of T_4 required to elicit electrically detectable activity in the erector spinae mass at T_4. The arrows indicate the incidence and direction of spread between all possible pairs of segments. The number on each arrow indicates the pressure applied to the spinous process of the segment from which the arrow originates, in order to initiate activity in the segment to which the arrow points. The absence of an arrow in one direction or the other between a given pair of segments indicates that evidence of spread was not obtained within the 7 kgm. range of the pressure meter. Thus, between a given pair of segments, dependent upon factors which are examined in subsequent sections, there may be no spread, one-way spread, or two-way spread.

lying the spinous process did not, in any case, affect the threshold. It appears, therefore, that important receptors or nerve endings for this reflex are closely related to the tip of the spinous process.

Correlation of Other Characteristics with Reflex Threshold. 1. *Pain.* Pain at the spinous process was not commonly produced in segments having high (H or N) reflex thresholds even with severe pressures beyond the range of the stimulator. When it did occur, it was fleeting and did not outlast the stimulus. In contrast, at L segments (and to a lesser degree at M segments) pain usually occurred well within the 7 kgm. range, and often outlasted the stimulus. Further, this low threshold pain was invariably reported as "different" and said to resemble that resulting from a "bone bruise".

2. *Post-traumatic soreness.* After repeated stimulation, the spinous process of an L segment often remained tender for more than 24 hours; this did not occur at H or N segments.

3. *Differences in physical characteristics of supraspinous tissues.* The differences in the palpable characteristics of the tissue overlying L and N spinous processes are marked, and detectable even by relatively inexperienced observers. The differences exist mainly in the skin and in the tissues which closely invest the vertebral spines. In the absence of reliable objective methods for the study of tissue texture, palpation was relied upon and description given in subjective terms. We believe the words "doughy" and "boggy" are the most descriptive of the texture and resiliency of the tissues overlying the spinous processes of low threshold segments. In high threshold areas, the tissues are not doughy and the bone outlines are sharp and hard. Differences in physical palpatory features of the adjacent muscles may also be detected. In low threshold areas the spinal extensors appear to possess less resiliency to pressure deformation than corresponding muscles in high threshold segments, and "ropy" and hyperesthetic bundles may often be identified.

The differences in physical characteristics among the tissues overlying the spinous processes of segments of different threshold were sufficiently characteristic and marked that palpation of these tissues by an experienced observer permitted fairly reliable prediction of pressure threshold groups (L, M, H, or N). When such predictions, made on the 4 selected segments on each of 10 subjects, by one of us (J. S. D.) were compared with the independently determined electromyographic findings the predictions were found accurate, with respect to threshold group, in 35 out of the 40 vertebrae palpated; the remaining 5 were off by only 1 threshold group.

4. *"Rest activity".* Normal muscle, completely at rest, is characterized by an absence of action potentials (1, 2–5, 7, 8, 10, 15–17).

For obtaining electromyograms of the spinal extensors, with the necessary needle contacts, the prone position provides optimal relaxation. Despite apparent relaxation of subjects "rest activity"[3] was frequently encountered at

[3] This term, used by Buchthal and Clemmeson (1) to designate activity in the absence of voluntary effort is to be preferred over "spontaneous activity", used in previous reports from this laboratory, since the latter may incorrectly imply that the activity originates at the motoneuron itself.

low or medium threshold segments. It was often necessary to position and reposition the shoulder girdle, upper extremities, head, and at times the lower extremities, or to calm the restless or apprehensive subject, in order to eliminate rest activity.

In our series four experiments had to be terminated because of persistent rest activity at one or more segments. Each of the four subjects at another time served in a successful experiment in which rest activity was eliminated.

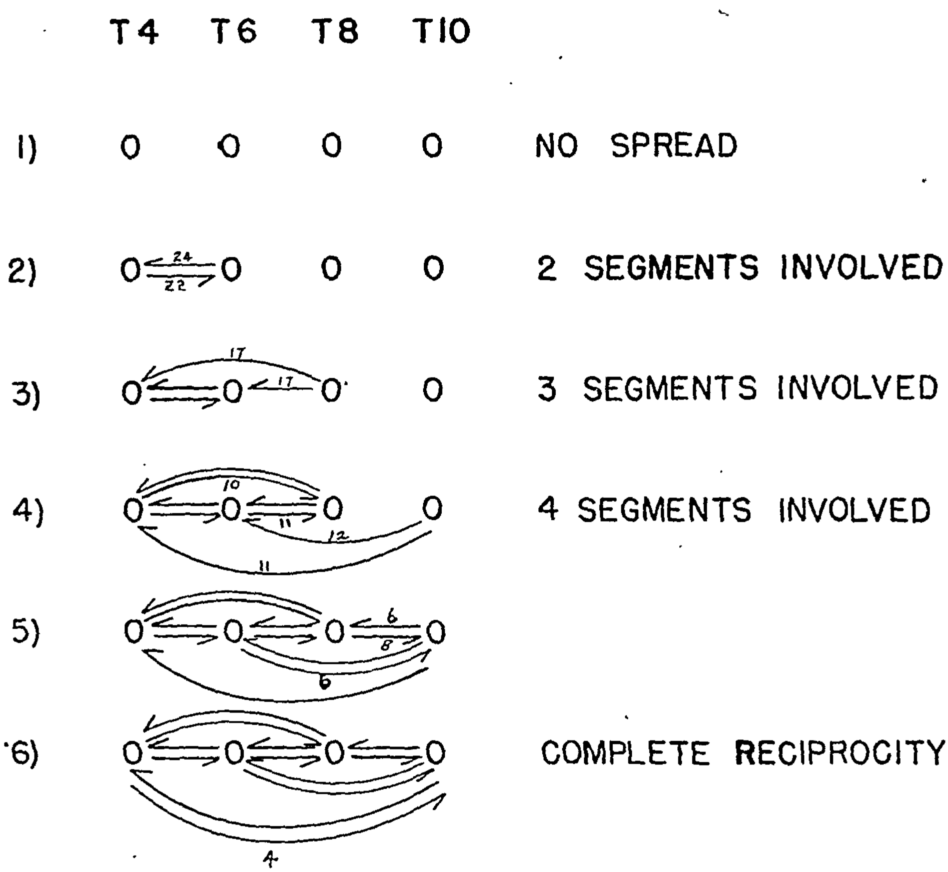

Fig. 2. Progression of spread. For explanation see text. The number on each arrow indicates the number of times that spread occurred in that direction between that pair of segments in the 30 experiments diagrammed in figure 1.

In all cases the persistent rest activity was found at L or M segments. One of the subjects, during the unsuccessful experiment, was perturbed by illness of his child; one was much more apprehensive than the average subject, and two walked with a limp, due to poliomyelitis in one and an old injury in the other.

Occasionally, one or more units in an L segment fired during inspiration or expiration. Usually this intermittent activity was not long lasting and could be eliminated by the same measures which were effective on ordinary rest activity.

Apparent "rest activity" was only very rarely encountered at H or N segments, and was always easily abolished by correction of some positional stress.

Thus, low threshold segments are apparently hyper-excitable, not only to pressure stimuli applied to the corresponding spinous process, but also, to impulses reaching them from higher centers and from proprioceptors.

Part II. In part I, observations have been presented concerning the response of segments of the erector spinae muscle to pressure stimuli applied to corresponding spinous processes. In this section observations are reported on the responses in muscle segments to stimulation of remote spinous processes, i.e., on observations of irradiation or intersegmental spread of excitation.

Spread Patterns. Incidence and distribution of spread among the four segments fell into five distinct patterns or groups (fig. 1).

1. *Order of spread.* There is a definite order to the frequency with which the different arrows appeared in the series of 30 experiments. We consider that these frequencies are an index of 1, the probability of spread in a given direction between a given pair, and 2, the order in which spread tends to appear in the progression toward "complete reciprocity" among the 4 segments. This progression and the frequencies are shown in figure 2.

2. *Segmental level.* The upper 2 segments participate in spread with significantly greater frequency than the lower two. The number of arrows (figs. 1, 2) to and from each segment were as follows:

T_4– 88; T_6– 92; T_8– 69; T_{10}– 47[4]

It will be noted (fig. 1) that when only 2 segments are involved in spread they are invariably the upper two (T_4 and T_6); when only 3 are involved, they are the upper 3.

3. *Segmental threshold.* Frequency of spread to and from a given segment is inversely related to threshold. No-spread pairs consist predominantly of H and N segments; two-way spread pairs consist predominately of L and M segments; and the one-way spread pairs exhibit intermediate thresholds.

4. *Segmental intervals.* (Distance between the members of a pair.) The incidence of spread declines with increasing distance between segments. The relation of thresholds and of segmental intervals to incidence of spread is shown in figure 3.

5. *Direction of spread.* Figure 2 shows that incidence of cephalic spread far exceeds that of caudal spread (except for a slight caudal predominance between T_8 and T_{10}). Of 32 cases of one-way spread (fig. 1) 29 are cephalic. Further, where there is two-way spread, the thresholds for cephalic spread are predominantly lower than those for caudal spread.

[4] Since T_4 and T_{10} are the terminal segments in the arbitrarily selected series of segments they are at a relative disadvantage as regards spread. However, were the series extended to include T_2 and T_{12}, segments T_6 and T_8, as well as T_4 and T_{10}, would also show increased participation in spread. T_4 would probably show the largest increase of all, certainly larger than that of T_{10}. The relative superiority of the upper segments of the series would remain.

Our analysis of the data indicates that preferential cephalic spread is an intrinsic feature of these reflexes and that the greater facility for cephalic spread exists regardless of whether the threshold of the superior segment (of a given pair) is lower than, higher than, or the same as that of the inferior segment.

In view of the fact that L's and M's are predominantly in the upper segments, this may be related to the intrinsic directional factor. Nevertheless,

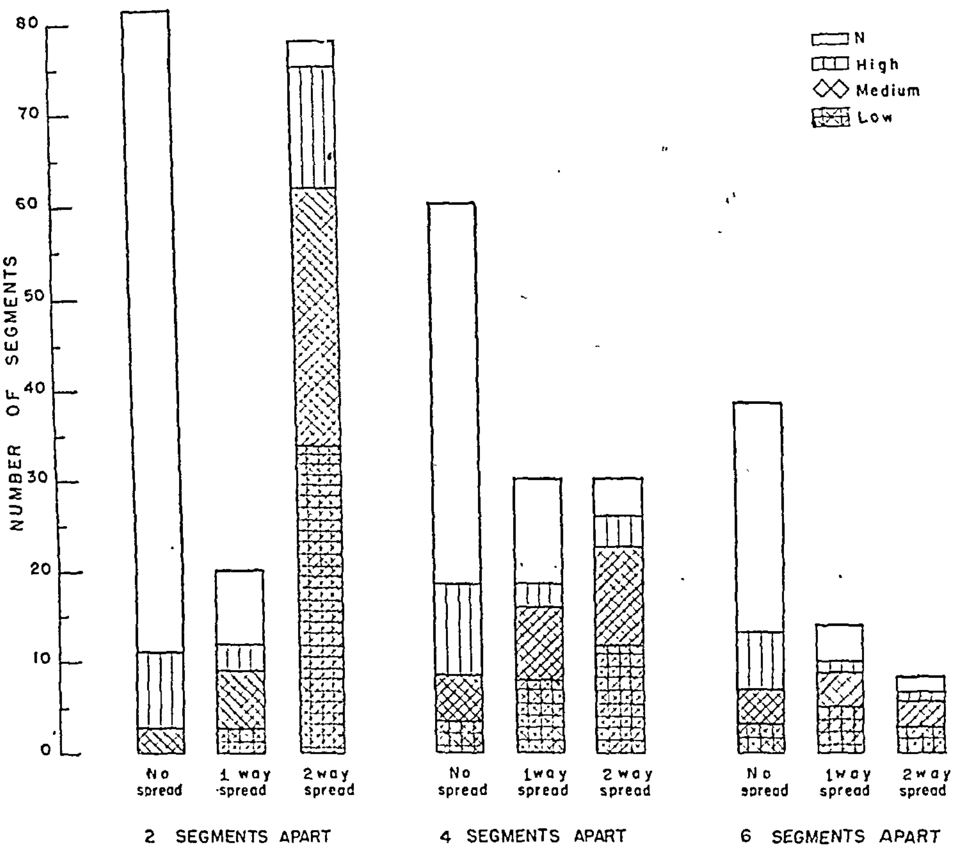

Fig. 3. Influence of distance and threshold on spread of excitation. Individual segments appear in the graph as many times as they can be paired, e.g., T_4 and T_{10} are paired with levels 2, 4, and 6 segments apart; and T_6 and T_8 twice with levels 2 segments apart and once with levels 4 segments apart. The cross-hatching code designates the number of segments in L, M, H or N categories. Thus, for example, of the segments showing no spread at a 2 segment interval, 4 were M, 7 were H, and 69 were N.

the fact remains that, in our experiments, the reflex muscular responses of L and M segments were relatively easily evoked by stimulation of the spinous processes of remote H or N segments; spread in the reverse direction was uncommon. Stimulation of the N spinous process not only failed to elicit responses from muscles of the same segment, but also those of (N or H) segments which intervene between it and the responding L segment. (Expts. 35, 22, 28, 36, fig. 1.)

Selective spread of excitation to L segments was further demonstrated by the application of slight tactile stimulation to remote areas of skin (e.g., shoulder or scapula) whereupon activity frequently appeared in L segments, but never in others.

6. *Procainization and spread.* It is of interest in this connection, although our observations are as yet few, that while procainization of the spinous process of an L segment raised the self-threshold beyond 7 kgm., the reflex responsiveness of that segment to stimulation of other spinous processes remained unchanged.

Discussion. These experiments confirm, in location and degree, that significant subject-to-subject and segment-to-segment differences in spinal reflex thresholds occur in the "normal" human.

The data indicate that differences in pressure thresholds reflect differences in central facilitation, and that the facilitation is due to a bombardment of the motoneurons by impulses originating, in part at least, from points other than the spinous process which was the site of stimulation. The evidence may be summarized as follows:

1. The L and M segment shows hyper-excitability to local and distant stimulation including that from N segments. Impulses from an N spinous process may bypass motoneurons in intervening high threshold segments to activate motoneurons in the ventral horn of a more distant L or M segment.

2. The effectiveness of pressure at the spinous process of N segments in eliciting activity from remote L or M segments is not ascribable to mere facilitation of continuous impulses from the spinous processes or supraspinous tissues of the L and M segments, since at least from our limited observations, the responsiveness of an L segment (to distant stimulation) is unchanged by procainization of the tissues closely investing its spinous process.

3. Spread *to* L segments is much more frequent than spread *from* L segments.

4. L segments are hyper-excitable to impulses other than those originating from external stimulation; "rest activity" is common in these segments.

5. Right and left sides, at the same level, may show strikingly different thresholds to pressure at the *same* spinous process.

Lloyd's studies (11, 12), with facilitating volleys, of the quantitative relationship between subliminal fringe and the discharge zone in the cat have led to his conclusion that (11) "It is unlikely therefore, that any significant number of motoneurons are close to or at threshold in the resting pool, for, if there were, the first afferent impulses to enter the pool should secure a postsynaptic discharge". His work demonstrated that a motoneuron pool, in the absence of a facilitating or test volley or both (to the dorsal root), is resting. This resting state represents a check on the far-flung interneuron system and, in effect, insulates the final common path against firing every time an afferent impulse reaches the pool.

While such a mechanism certainly exists in man and while Lloyd has demonstrated it in experimental preparations where complete control of facilitating, inhibitory and test volleys can be maintained, our observations indicate that

in "normal", intact man it is possible to have quite a different situation. Not only may different pools, in close anatomic proximity, show different (and constant) degrees of closeness to threshold but, indeed, certain pools may be at, or above, threshold (rest activity) in the absence of external stimulation. Since Lloyd has demonstrated that a considerable portion of the cells in a motoneuron pool must be in a state of subliminal excitation before discharge from that pool occurs, it seems apparent that reflex thresholds (measured by the pressure meter) are a measure of the size of the subliminal fringe or of the degree of facilitation maintained at a given spinal segment. Thus a 1 kgm. segment has such a large subliminal fringe that relatively few additional impulses reaching it (from any source) will extend it into the discharge zone.

In addition to the demonstration of central facilitation, correlation was found among *a*, the reflex threshold; *b*, the palpable characteristics of supraspinous tissues; *c*, the susceptibility of those tissues to lasting soreness following minor trauma, and *d*, the pain threshold (and pain characteristics); the basis for this correlation has not yet been learned.

An attractive possibility which might account for this relationship is that in a given segment there are pools of neurons other than anterior horn cells that are also facilitated and that their hyper-activity, through trophic, vasomotor or other influences, produces the observed changes in the tissue.

The lower resistance to minor trauma and to painful stimuli may be secondary to the tissue alterations. Certain similarities to the nocifensor tenderness described by Lewis (9) are indicated. The possibility remains, however, that the lowering of the pain threshold may also be due, in part at least, to central facilitation.

The facilitation indicated in the low threshold pool may explain what Mackenzie (13, 14) referred to as exaggerated responses in an area of "irritable focus" and, by direct evidence, establishes the latter at either the interneurons, the motoneurons, or both. Hinsey and Phillips (6), in connection with referred pain, have also translated the "irritable focus" into terms of facilitation.

The final question to be asked in connection with the present observations is concerned with the origin or origins of the impulses which facilitate the low threshold pools. Several sources suggest themselves: the higher centers, viscera, proprioceptors (i.e., joints, tendons, ligaments or muscles). In these experiments, however, the high degree of constancy and especially the high degree of localization—to one or two segments, the frequent differences in threshold of right and left side of the same segment, and the absence of psychoneurotic and visceral symptoms in our subjects would seem to rule out the first two as major sources.[5] We are inclined to believe that the facilitating impulses arise from segmentally related structures.

[5] This is not to deny that psychogenic or viscerogenic impulses may significantly affect segmental thresholds. Our own experiments demonstrate that apprehensiveness, anxiety, transient illness, etc., may cause widespread lowering of thresholds. In our subjects these influences appear to have been superimposed on the primary, and more constant, factors influencing thresholds.

SUMMARY

1. The reflex responses of the erector spinae muscles to measured pressure applied to the spinous processes at selected spinal segments were studied. The existence of constant differences in reflex thresholds of segments in different subjects, and from segment to segment and from side to side in the same subject, has been confirmed.

2. Low threshold segments showed reflex hyper-excitability to pressure upon the corresponding spinous processes, to pressure upon the spinous processes of distant, high threshold segments, and to impulses from proprioceptors associated with positioning, from remote areas of skin and from the higher centers.

3. It is concluded that low threshold segments are those in which a relatively large portion of the motoneurons are maintained in a state of facilitation due to a chronic bombardment by impulses from some unknown source. Presumptive evidence indicates that the facilitating impulses arise from segmentally related structures.

4. Correlation of motor reflex threshold with *a*, pain thresholds; *b*, susceptibility of supraspinous tissues to minor trauma, and *c*, with tissue texture, has been demonstrated. This suggests that neurons other than the motoneurons in the low threshold segments may be simultaneously facilitated.

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BLOOD-BORNE VASOTROPIC SUBSTANCES IN EXPERIMENTAL SHOCK[1]

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Our studies on the peripheral circulation of animals subjected to hemorrhagic and traumatic shock have emphasized the development of characteristic disturbances in the terminal vascular bed. These disturbances could be classified into two categories: an initial, hyper-reactive response which is compensatory, and a subsequent, progressive impairment of the vascular responsiveness, which is decompensatory. The precise nature of these disturbances and their relative ease of detection have offered a means of determining progressive changes in the shock syndrome in a manner hitherto impossible. Moreover, by injecting small quantities of blood of the shocked animals into test rats, it was found possible to use these criteria to determine the presence of humoral substances in the blood. A preliminary report of this has been published (1).

Cannon (2), Bayliss (3), Moon (4) and others had proposed the concept that the blood carries principles which either initiate or contribute to shock conditions. However, no one has hitherto been able to substantiate this concept because the criteria used, such as blood-pressure changes and survival, have been too gross to serve as evidence. Attempts to produce shock in a normal animal by replacing its blood with that of an animal in shock have not clarified the problem. Such a procedure involves the removal of large amounts of blood from both donor and recipient and this introduces complications which defeat the purpose of the experiment.

The present contribution presents a detailed analysis of the occurrence of two types of humoral principles in the blood of animals subjected to a variety of experimentally induced shock procedures.

The findings are based on a technique which involves the responsiveness of the terminal arterioles and precapillaries to the topical application of epine-

[1] This work was done under contract, recommended by the Committee on Medical Research between the Office of Scientific Research and Development and New York University. It was also supported by Eli Lilly and Co. and the Josiah Macy Jr. Foundation.

It constitutes the tenth of a series of studies on experimentally induced shock. The first study is listed as no. 5, the second as no. 9, the third as no. 10, the fourth as no. 7, the fifth as no. 8, in the references of the present paper. The sixth on the rat tourniquet is in Ann. Surg. **120:** 791, 1944; the seventh on anesthesia, in Surgery **18:** 48, 1945; the eighth on tourniquet shock, in Surg., Gynec. and Obstet. **80:** 593, 1945 and the ninth on anesthesia, in Anaesth. **6:** 362, 1945.

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[3] Other individuals participating in the study were C. G. Grand, C. Hyman, M. J. Kopac, M. E. Krahl, R. E. Lee.

phrine. The method of assaying the vasotropic activity of any given blood sample consists of injecting small quantities of the blood into a rat and observing the effect on the blood vessels of the exposed meso-appendix. The method has two unique features. First, the amount of blood needed for the tests is so small, that it can be taken at repeated intervals from the donors without unduly influencing the oligemic aspects of the shock syndrome. Second, the reactions of the capillary circulation in the test rat are highly selective and serve as extremely sensitive criteria for detecting the presence of vasotropic principles.

The investigations described in this paper are based on criteria of structure and functional activity of the peripheral vascular components already established in detail (5) for a variety of tissues in a large series of normal animals. It is necessary to recite here two main features of the normal circulation. One is vasomotion, a periodic dilatation and contraction of the terminal arterioles, metarterioles and precapillary sphincters. The other is the existence of a precise level of reactivity which the muscular components of the normal capillary bed show to epinephrine. These are the features which exhibit characteristic alterations in the course of the development of the shock syndrome..

Two types of reactions could be distinguished, a vasoexcitor and a vasodepressor, of which one, and later, the other were found to predominate during the progressive stages of the syndrome. The participation of these reactions in the shock syndrome serves to explain many of the phenomena which lead to and culminate in the collapse of the peripheral circulation. More recently (6) it has been demonstrated that the vasoexcitor principle (VEM) has its origin in the kidney and the vasodepressor (VDM) in the liver and in traumatized skeletal muscle.

Methods. During the course of studies over a period of years in this laboratory, shock was produced by a variety of procedures in a total of about 600 animals. These included 170 dogs and 50 rats which were subjected to hemorrhage, acute and graded (7, 8); 46 dogs, 10 cats, 150 rabbits and 75 rats subjected to hind-limb tourniquet (9, 10); 150 rats subjected to trauma in the Noble-Collip drum (11); and 9 dogs subjected to leg pounding. The last were obtained through the co-operation of Dr. M. I. Gregersen and Dr. W. S. Root of the College of Physicians and Surgeons, Columbia University.

Young rats, weighing from 100 to 150 grams were used for testing the vasotropic activity of blood samples. They were anesthetized by intramuscular injection of sodium pentobarbital (3 to 4 mgm. per 100 grams of body weight). The majority of the test rats were prepared only for observing the capillary bed of the meso-appendix. Some were mounted for simultaneous observation of the circulation in the interdigital web under one microscope and in the exposed meso-appendix under a second. The interdigital web was used chiefly for recording the blood pressure by means of a specially constructed, pneumatic inflation cuff around the thigh (12). The record was obtained by noting the amount of pressure required to interrupt the blood-flow through the arterial vessels in the skin of the interdigital web.

The method for exposing the meso-appendix is given in a previous paper (5). Essential precautions were a minimal handling of the cecum while draping the meso-appendix over a glass horseshoe on the stage of the microscope and a rigid maintenance of the exposed tissue at body temperature and in constant moisture conditions by means of a drip of warm, Ringer's solution containing one per cent ash-free gelatin.

Two vascular criteria were routinely used during the assay technique. One was a change in the responsiveness of the arterioles and precapillaries to the topical application of epinephrine. The other was a disturbance in the intermittent contraction-relaxation activity (vasomotion) of the metarterioles and precapillaries the cycle of which normally varies between 1 to 3 minutes. The basic rôle of these two mechanisms in maintaining the capillary circulation is indicated by the fact that disturbances in them are immediately reflected by changes in blood-flow through the capillary bed.

The accuracy of the observational method depends upon rigid adherence to a procedure for maintaining the exteriorized tissue in a physiologic state of reactivity. Under such conditions the normal reaction of the vessels to epinephrine remains at a constant level for at least 2 to 3 hours. Emphasis must be placed on the selection of vessels best suited for the test. The terminal arterioles or metarterioles and the sphincteric, precapillary branches have been found to be the most highly reactive components of the bed. The preciseness of response of these vessels to epinephrine serves as a specific end-point which can be quantitated for the comparative analysis of different blood samples. Neighboring vessels in the same capillary bed have their own basic threshold levels of reactivity. A necessary precaution, therefore, is to make all the observations for a given test only on those vessels whose normal reactivity has been previously determined. The *minimal threshold* of reactivity of the selected vessels is determined by making successive topical applications of increasing concentrations of epinephrine until a concentration is reached which induces only a narrowing for several minutes just sufficient to occasion a slowing but not an interruption of the flow of blood through the network of capillaries fed by the affected vessel.

For each test the epinephrine is freshly prepared from a 1/100,000 stock solution. The dilutions are made with gelatin-Ringer in a 1 cc. tuberculin syringe. The vascular reaction is observed by temporarily turning off the drip of the gelatin-Ringer which bathes the mesentery, and applying 3 to 4 drops of the epinephrine solution to the surface of the mesentery.

In the normal rat, under pentobarbital anesthesia, the threshold concentration of epinephrine was found, in different individuals, to vary between one part in one million to one part in six million. Animals were discarded when the response to epinephrine was so poor as to require concentrations greater than 1 part in one million to produce vasoconstriction.

Normally, the vessels continue for several hours to show a uniform response to the initially determined minimal effective concentration of epinephrine. When, following the intravenous administration of a test sample, any per-

sistent fluctuation was found to occur above or below this initial reactivity, the intensity and duration of the effect served as a means for quantitating the humoral vasotropic activity of the sample. These vascular effects in the test animal were temporary, a feature which distinguished them from non-specific toxic effects. By using a statistically significant number of test animals, it was possible to quantitate the effect of the humoral principle present.

Collection and preparation of blood samples for testing. Blood samples were collected in 1 to 5 cc. amounts. During the early phases of the work the blood was allowed to clot and the serum kept in a refrigerator for 24 hours to allow for the disappearance of vaso-tonic effects which sometimes occur with freshly prepared, normal serum. Later it was found that heparinized plasma was equally satisfactory and could be tested immediately.

Classification of the blood samples. Prior to the injection of the test samples one or several arteriolar precapillary units in the meso-appendix were selected for observation. The *amplitude* and *rate* of their vasomotion was noted and the minimal effective concentration of epinephrine determined.

The serum or plasma (0.5 cc.) was then injected into the tail vein. Normal samples produced no changes in the vascular criteria being studied. With active samples, the vascular effects generally appeared within two to three minutes and persisted for as long as twenty to forty minutes. Some of the samples produced changes in blood-pressure corresponding with the vaso-excitor or depressor effects observed in the capillary bed. However, in most cases, the blood-pressure changes were not sufficiently constant to serve as a routine index of vasotropic activity.

Three categories of activity were recorded:

1. *Neutral.* These produce no demonstrable change in the reactivity of the capillary vessels. The amplitude and rate of vasomotion and the concentration of epinephrine needed to produce a minimal effective constrictor response remain the same as prior to the injection.

2. *Vasoexcitor.* These enhance the reactivity of the muscular components of the capillary bed. The specific reactions in the meso-appendix are as follows: (a) Augmentation in amplitude and rate of the vasomotion, especially of the precapillaries, with an ensuing restriction of circulation in the bed and a by-passing of many of the capillaries; (b) heightening of the epinephrine response, the vessels reacting to dilutions as high as 1 part in 30 to 40 million (a 10 to 20-fold change from the normal value).

3. *Vasodepressor.* These lower the epinephrine reactivity, depress vasomotion and in some instances lower blood pressure. The following changes are observed in the meso-appendix. (a) There occurs a diminished reactivity to epinephrine of the arterioles and precapillaries so that the minimal effective concentrations characteristic for the control period now produce no evident vasoconstriction or slowing of the capillary circulation. Serum samples, having marked vasodepressor properties, frequently abolish all response to normal threshold concentrations of epinephrine. (b) There occurs a diminution in frequency of vasomotion of the metarterioles and precapillaries. In many

cases the vasomotion remains suppressed for as long as 30 to 40 minutes whereupon the flow through the capillary bed becomes pronouncedly slowed, especially in the muscular venules. (c) In some instances there was a fall in blood pressure to 10 to 20 mm. below normal levels.

EXPERIMENTAL DATA. *Blood of rabbits in tourniquet shock.* Over 100 rabbits, anesthetized with intravenously administered sodium pentobarbital (20 mgm./kgm. body weight), were subjected to a tourniquet of one hind-limb for 5 to 6 hours, both arterial and venous flow being completely occluded. The ligation was done as high as possible, care being taken not to thrombose the femoral vessels. The animals generally recovered from the anesthetic and were alert for about 2 hours before the tourniquet had been released. When the tourniquet was removed shock developed only in those animals in which the limb warmed up readily and underwent a progressive swelling.

Blood samples were taken from the femoral vein of the undamaged limb or by cardiac puncture. A control sample was taken before applying the tourniquet; a second, prior to its release; and subsequent samples during the development of shock after release of the tourniquet. In 77 animals, shock-blood for assay was taken only terminally. In 23 animals the samples were taken periodically during the course of the shock. The rat-test data on these samples are given in table 1. One case is included in which the tourniquet occlusion was not complete, so that a certain amount of swelling of the leg occurred while the tourniquet was still on.

In table 1, the first four rabbits listed did not go into circulatory collapse, although the injured limbs underwent considerable swelling. They were sacrificed 8 to 10 hours later. The remaining nineteen rabbits developed shock symptoms and died from 1.5 to 5 hours after the release of the tourniquets. In these rabbits the swelling of the limb was accompanied by the development of a rapid, thready pulse and an irregular, shallow abdominal respiration. As the shock deepened, the rabbits no longer responded to painful cutaneous stimuli, showed a poor corneal reflex and progressively went into a comatose state from which they did not recover.

Blood samples taken before the application of the tourniquet gave a neutral test reaction. Blood samples taken while the tourniquet was in place gave neutral responses with one exception, no. 5, in which a vasoexcitor response was obtained. Apparently this was a result of the poorly-tied tourniquet which permitted swelling of the limb during the period of occlusion. In all cases the blood samples were found to be vasoexcitor during the period of swelling of the hind limb.

The blood samples of the four rabbits which survived did not give vasodepressor test reactions: The persistence of a vasoexcitor reaction of the blood sample invariably indicated eventual survival.

In the majority of the fatal cases, the blood samples were vasoexcitor during the first hour and subsequently became vasodepressor at 1.5 to 3 hours after tourniquet release. Three of the rabbits (nos. 21, 22 and 23) showed a vasodepressor reaction as early as 30 to 60 minutes after release of the tourniquet.

The following are representative rat-test protocols of two blood samples: one taken during the period of impending shock and the second after profound shock had developed.

Thirty minutes after tourniquet release (femoral vein blood, rabbit 10). Meso-appendix of rat (150 grams) exposed and metarteriole with precapillary selected. The epinephrine reactions are indicated in figure 1. The minimal effective concentration of epinephrine during control period was 1:2 million. The injection of serum sample into the tail vein was followed by a transient speeding of capillary flow. At three minutes, the response to the applied epinephrine

TABLE 1

Rat meso-appendix assay of serum of rabbit in tourniquet shock

RABBIT NO.	PRIOR TO AND DURING TOURNIQUET	TIME IN HOURS AFTER RELEASE OF TOURNIQUET							FATE
		0.5	1.0	1.5	2.0	2.5	3.0	4.0	
1	N	E			E		E		Survived
2	N						E		Survived
3, 4	N		E					N	Survived
5	N E		e		D		D		Died in sixth hour
6	N		E				D		Died in sixth hour
7	N				D	D			Died in fourth hour
8, 9	N						D		Died in fifth hour
10	N	E				D			Died in sixth hour
11	N		E			D			Died in sixth hour
12, 13	N					D			Died in sixth hour
14, 15	N					D			Died in third hour
16	N		E		D				Died in fourth hour
17	N			D		D			Died in sixth hour
18	N		E			D			Died in fifth hour
19	N	E	E	D					Died in fifth hour
20	N				D				Died in sixth hour
21	N		D			D			Died in third hour
22	N	D		D					Died in second hour
23	N	D							Died in second hour

Legends: N = neutral, E = vasoexcitor, e = mild vasoexcitor, D = vasodepressor

increased and reached a peak at 15 minutes when the mimimum, effective concentration was found to be 1:8 million (a four-fold increase over the control). During this period the constrictor phase of the vasomotion of the precapillary sphincters became augmented until, at 15 minutes, there occurred a considerable reduction of capillary flow. At 35 minutes the epinephrine reactivity had returned to normal. The sample was classified as a 35 minute vasoexcitor.

Two and a half hours after tourniquet release (heart blood, rabbit 10). The epinephrine reactions are indicated in figure 2. The vessels in the meso-appendix of a 165 gram rat responded to a minimal effective concentration of epinephrine of 1:1.5 million. Two minutes after injection of the blood sample, the response to epinephrine had become less marked, and, at 6 minutes, the application

of 1:1.5 million of epinephrine produced no response. Vasomotion became less evident and was completely suppressed at 10 to 18 minutes, at which time the circulation in the capillary bed was appreciably slowed. The blood pressure fell from 105 to 90 mm. Hg within 3 minutes after the injection of the shock

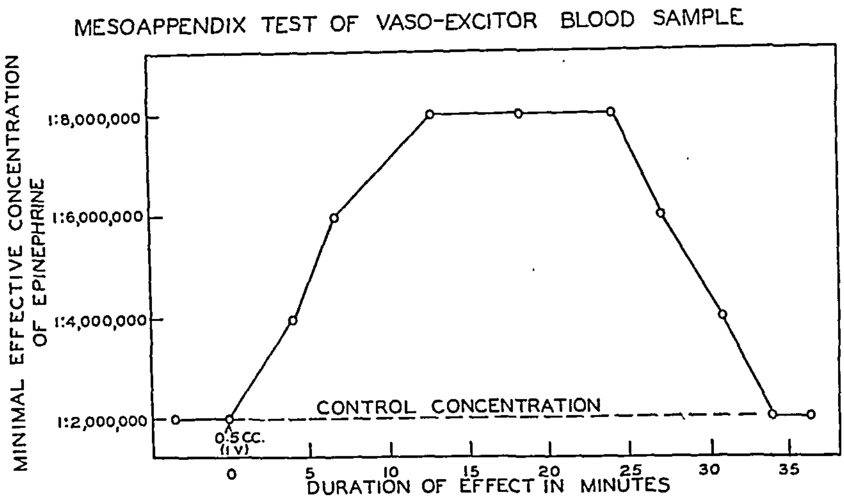

Fig. 1. Mesoappendix test of vaso-excitor blood sample taken at 30 minutes from rabbit 10 during the early compensatory period of shock following tourniquet occlusion of one hind limb.

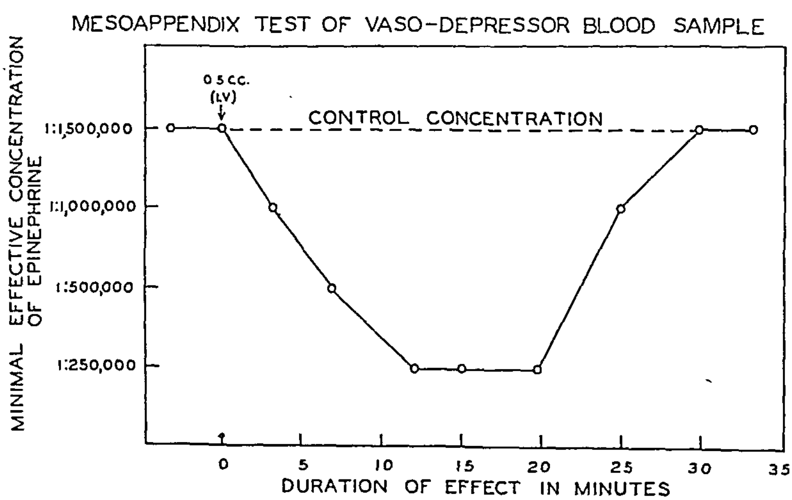

Fig. 2. Mesoappendix test of vaso-depressor blood sample taken at 2½ hours from rabbit 10 during the decompensatory period of shock following tourniquet occlusion of one hind limb.

blood. At 30 minutes the reactions had returned to normal. This sample was classified as a 30 minute vasodepressor.

Blood of dogs in tourniquet shock. Twenty dogs were used, fifteen under pentobarbital anesthesia and five under morphine sulfate. Blood samples for testing were taken from the jugular vein.

Rubber tourniquets were placed on the thigh as far up as possible so as to occlude both arterial and venous flow, care being taken not to damage the femoral vessels. Of dogs under pentobarbital anesthesia, it was necessary to occlude both hind limbs in order to produce fatal results. When morphine was used the animals were temporarily placed under ether anesthesia before applying the tourniquets. In these cases occlusion of only one hind leg was sufficient. With the use of morphine sulfate alone Fine and coworkers (11) found that occlusion of two hind legs for at least 12 hours was necessary. This difference from our findings is probably due to the predisposing effect of ether, the deleterious action of which in hemorrhage shock we have previously described (12).

Morphine sulfate. The morphine (2 mgm./kgm.) was injected intravenously and, thirty minutes later, ether was administered just sufficient to permit application of a tourniquet to one hind leg. At the end of 8 hours the tourniquet was removed. In all five dogs the injured limbs swelled pronouncedly and the dogs went into fatal shock.

Blood samples were obtained prior to and at intervals after release of the tourniquet. Those obtained prior to release gave neutral test reactions. During the period of swelling of the limb the blood samples were vasoexcitor. The swelling reached a maximum within an hour and samples taken 2 hours or more after release of the tourniquet, were found, in the case of four of the dogs, to be vasodepressor; in the case of the fifth, the successive samples changed from a vasoexcitor to a neutral test reaction prior to death.

Pentobarbital. Sodium pentobarbital (30 mgm/kgm.) was injected intravenously and tourniquets applied to both hind limbs for periods of from 6 to 12 hours. The majority of the dogs were alert 2 to 3 hours after applying the tourniquets and gave no evidence of pain. In a few cases it was necessary to give an additional intramuscular injection of pentobarbital (1-15 mgm./kgm.). After removal of the tourniquet 13 of the 15 dogs went into fatal shock and collapsed 3 to 7 hours later. The maximal swelling of the limbs occurred during the first 2 hours. During this period blood samples were vasoexcitor. After the maximal swelling had been reached, the reaction of the subsequent blood samples became neutral and then became increasingly vasodepressor as the shock deepened. The two dogs which did not go into shock exhibited no swelling of the limb, presumably because of thrombosis of the femoral vessels. The blood of these dogs remained neutral during the five hour period in which they were observed.

Shock induced in dogs by leg pounding. Blood samples were obtained by Doctor Gregersen and Doctor Root from nine dogs subjected to traumatic shock by leg pounding.[4] Heparinized blood was taken from the femoral artery before

[4] The technique, described by Doctor Root, is as follows: Under surgical ether anesthesia, the thigh muscles of both hind legs of the dog were bruised by blows with a light raw-hide mallet delivered 700 to 1000 times (for a 15 kgm. animal) over a 15 to 20 minute period. The skin was not perforated, no large vessels were ruptured and no bones were broken. Marked swelling of the traumatized tissues rapidly ensued and a majority of the dogs died in shock between 3 to 9.5 hours after the termination of the trauma.

and at varying intervals after traumatizing the limbs. The samples were chilled and transferred within 24 to 48 hours to our laboratory for testing. The results as compared with the blood pressures and overall diagnoses made by Doctor Gregersen and Doctor Root are summarized in table 2. Their diagnosis of the state of shock was based (13) on changes in blood pressure, respiration, heart rate, body temperature, appearance of mucous membrane and signs of central nervous depression.

TABLE 2

DOG NO.	NO.	BLOOD SAMPLES TIME TAKEN*	BLOOD PRESSURE*	STATE OF SHOCK*	RAT MESOAPPENDIX ASSAY	FATE OF DOG
			mm. Hg			
I	1	Before trauma	108	—	Neutral	
	2	48′ after trauma	75	Moderate hypotension	Vasoexcitor	
	3	$1^h33'$ after trauma	57	Mild shock	Mild vasodepressor	
	4	$1^h42'$ after trauma	55	Mild shock	Mild vasodepressor	
	5	$2^h4'$ after trauma	50	Deep shock	Vasodepressor	
	6	$2^h24'$ after trauma	50	Deep shock	Vasodepressor	
	7	$4^h9'$ after trauma	25	Profound	Marked vasodepressor	Died at 4½ hrs.
II	1	Before trauma	113	—	Neutral	
	2	$4^h15'$ after trauma	38	Deep shock	Marked vasodepressor	Died
III	1	Before trauma	108	—	Neutral	
	2	$4^h30'$ after trauma	44	Deep shock	Vasodepressor	Died
IV	1	Before trauma	119	—	Neutral	
	2	$5^h25'$ after trauma	40	In shock	Vasodepressor	Died
V	1	Before trauma	122	—	Neutral	
	2	5^h after trauma ($2^h30'$ after transfer)	82	In deep shock	Vasodepressor	Died
VI	1	Before trauma	104	—	Neutral	
	2	$5^h30'$ after trauma (2 hrs. after transfer)	84	Mild depression	Mild vasodepressor	Survived
VII	1	Before trauma	100	—	Neutral	
	2	$6^h20'$ after trauma	70	Moderate shock	Mild vasodepressor	Survived
VIII	1	Before trauma	97	—	Neutral	
	2	6^h after trauma (3 hrs. after transfer)	118	Slight shock	Mild vasodepressor	Survived
IX	1	Before trauma	114	—	Neutral	
	2	2^h after trauma	120	Early shock	Vasodepressor	
	3	6^h after trauma	130	Moderate shock	Vasodepressor	Survived

*These columns present data obtained by Gregersen and Root.

Twenty-four samples in all were tested. Nine samples gave neutral reactions. These were taken from the dogs prior to being subjected to trauma. One of the samples was vasoexcitor. This was from dog I taken at 48 minutes after trauma. Five of the samples were mildly depressor. Of these, three were taken at 5 to 6 hours after trauma from dogs VI, VII, VIII, which survived; while two were taken at 1½ and 1¾ hours from dog I which ultimately died but which, at the time the samples were taken, was still alert with a blood pressure of 85 to 80 mm. Hg.

The remaining nine of the samples were definitely vasodepressor. These were taken at 2 to 5 hours after the trauma of dogs I, II, III, IV, V and IX, all of which with the exception of no. IX, died.

Thus, in the dogs with the exception of no. IX, the vasotropic properties of the blood samples proved to indicate closely the state of shock of the dog at the the time when the sample was taken.[5]

Blood of rats traumatized in the Noble-Collip drum. With this method no anesthesia was used. The rats, with their front and hind feet tied together were exposed to repeated falls within a drum 2 feet in diameter, rotated at 45 turns per minute. Rats, subjected to 600 to 800 revolutions, died at varying intervals up to 20 hours after removal from the drum. For 650 revolutions the average mortality was 2 to 4 hours. Animals were excluded from consideration which suffered obvious intracranial or gastro-intestinal hemorrhage and died within 30 minutes after removal from the drum.

A description of the method and the circulatory changes during the shock syndrome has been published (11). In general, the symptoms obtained were lowered blood pressure, increased hemoconcentration and reduced blood volume.

TABLE 3

NO. OF RATS	TURNS OF DRUM	TIME AT WHICH BLOOD SAMPLES WERE TAKEN AFTER REMOVAL	REACTION WITH MESO-APPENDIX TEST	FATE
8	600–800	40–70 min.	vasodepressor	died
1	400	50 min.	mild vasodepressor	survived
2	350–400	45–60 min.	neutral	survived
1	300	90 min.	vasoexcitor	survived

The rats showed intense pallor, irregular respiration and poor response to painful stimuli.

Blood samples for assaying were taken from 12 rats by intracardial puncture at intervals varying from 40 to 90 minutes after the rats had been removed from the drum, as shown in table 3.

All the rats exposed to fatal trauma in the drum developed vasodepressor material in their blood. It was observed that with this type of shock the vasodepressor blood samples also produced an abnormal sticking of leucocytes in the capillaries and venules in the meso-appendix of the test rat.

Blood from dogs and rats subjected to hemorrhage. Two types of hemorrhage were studied: acute, in which an excess of blood was withdrawn within 1 to $1\frac{1}{2}$ hours; and graded, in which the blood loss was spread out over an extended period of time. Animals, subjected to acute hemorrhage, responded favorably to fluid replacement up to 5 to 10 minutes of expected collapse. Animals,

[5] These experiments were carried out soon after we had worked out the details of the rat meso-appendix technique in 1943, and had as their main purpose to check the reliability of this method of assay. The samples were delivered to us labelled by code numbers.

subjected to graded hemorrhage, became increasingly refractory to recovery by blood replacement therapy.

Acute hemorrhage. Over 75 rats and 25 dogs were used. Blood samples were withdrawn within 5 to 10 minutes after the onset of bleeding and, thereafter, at intervals throughout the syndrome up to shortly before death.

Unanesthetized rats were bled, some by heart puncture and others by immersing the cut stump of the tail in warm isotonic sodium oxalate in a graduated cylinder, for measuring the amount of blood loss. The dogs were anesthetized with intravenous injections of morphine (2 to 12 mgm./kgm.) or pentobarbital (30 mgm./kgm.).

The blood samples taken throughout the syndrome in both rats and dogs were uniformly vasoexcitor. In some instances the samples produced a transient rise in the blood pressure of from 10 to 15 mm. Hg above the normal.

Graded hemorrhage. In the course of the two years of our study on irreversible shock about 100 dogs were used and more than 450 blood samples were tested. The dogs were routinely anesthetized with pentobarbital or with morphine. The type of graded hemorrhage was one in which the dogs finally become irreversible to restoration of the blood previously withdrawn (4). Dogs under pentobarbital anesthesia were found to be more susceptible to bleeding and to the adverse effects of the prolonged hypotension than were dogs under morphine.

Blood samples, taken within 1 to 3 hours after the initiation of the bleedings, were markedly vasoexcitor. During the first hour the samples consistently produced an increase in epinephrine reactivity, accentuated vasomotion, and raised blood pressure of the test rat by 7 to 15 mm. Hg. With samples taken after the first hour of shock, the three reactions were lost successively in the following order: first, the blood pressure effect; second, enhancement of vasomotion and, lastly, at about the third to fourth hour, the epinephrine excitor response.

Samples taken after the onset of profound hypotension, especially during the so-called irreversible stage, were consistently vasodepressor. The greatest vasodepressor effects were obtained with samples taken during the last hour before death.

DISCUSSION. The results presented in this paper on the detection of vasotropic principles in the blood of animals subjected to shock are all based on the reactivity imposed on the terminal blood vessels in the meso-appendix of normal rats by the injection of blood samples. Changes in intensity and duration of the reaction of the blood vessels to the topical application of epinephrine served as an index for a semi-quantitative assay of the humoral substances.

Two major features present themselves during the development of the state of shock. One is the appearance of a vasoexcitor material (VEM) at the time when fluid is being lost from the circulation. The other is the gradual displacement of the VEM by an antagnostic vasodepressor material, the VDM.

In hemorrhagic shock, the VEM appears early in the blood after the loss of as little as 1 per cent of the body weight, an amount usually required to initiate slowing of the splanchmic flow as evidenced in the mesentery or the omentum. This material continues to be elaborated during acute hemorrhage almost to the

point of complete exsanguination, i.e., within 1–2 hours in dogs. The non-deleterious character of the VEM is indicated by the fact that replacement of the blood lost results in disappearance of the material and is followed by complete recovery of the animal. The vascular reactions are of a compensatory nature.

The elaboration of a vasodepressor principle (VDM) occurs only after a prolonged period of severely reduced peripheral circulation and consequent anoxia. Such a reaction occurs when the fluid loss (whole blood in hemorrhage and in leg pounding, plasma in tourniquet shock) is insufficient *per se* to precipitate circulatory collapse. Under this condition the animal continues to live for a protracted period with its tissues in a state of increasingly severe anoxia.

The significance of the vasodepressor aspect of the syndrome has been discussed in previous publications in which it was shown that the preponderancy of the VDM is associated with the onset of irreversibility to fluid replacement therapy (8, 9, 10).

In the experiments cited in this paper the appearance of marked vasodepressor material in the blood is almost invariably a prognostic indication of fatal circulatory collapse. This was demonstrated in different types of experimentally induced shock, viz., graded hemorrhagic, tourniquet and leg-pounding shock, and shock induced by the Noble-Collip drum. In addition, the development of the depressor material evidently is not limited to any one species of animal, since we have detected it in the blood of rats, rabbits and dogs. This gives to these principles a fundamental significance to the shock problem as a whole.

Concerning the reliability of the rat meso-appendix test for detecting the vasoexcitor and vasodepressor substances, it is significant that the reactions observed in the test animal are identical with those observed in the shocked animal at the time that the blood sample is withdrawn. Early in the development of the technique reliance was placed on a variety of overall changes in the capillary bed, such as changes in vasomotion, in number of capillaries containing an active blood flow, in the rate of the blood flow especially in the venules, etc. Later it was found that the reaction to an arbitrarily applied substance such as epinephrine could be used as a quantitative index of the vasotropic activity of the sample being tested. When the vasoexcitor principle predominates the vascular musculature becomes hyper-reactive; when the vasodepressor principle predominates the musculature becomes refractory and hypo-reactive to epinephrine. The epinephrine reaction has been extremely useful for assay purposes. The question remains open as to whether it is the specific reaction which is affected during shock in the intact animal.

The consistency in the appearance of the VDM with the development of irreversibility and fatal shock has been stressed in our previous publications and points to something more than a chance relationship between the humoral factor and the circulatory changes leading to fatal collapse. Evidence for a causal relation was found in experiments in which dogs, during the depressor phase of the shock syndrome, were infused with large amounts of blood (3-4 per cent body weight). The infusion induced a transitory elevation of the blood pressure to normal levels and a speeding of the peripheral blood flow in the

omentum. The subsequent fall of blood pressure was always *preceded* by a deterioration of the capillary circulation in the omentum. The fact that the slowing of the capillary flow occurs well in advance of any fall in blood pressure indicates that the arteriolar effects may very well be the cause of the blood pressure fall rather than a result.

The elaboration of vasodepressor material is probably not the only factor concerned with the collapse of the shocked animal. Other factors, neurogenic, etc., have been shown (14, 15) to participate in various types of shock. However, the vasodepressor factor is the only one which thus far has been directly related to the observed vascular phenomena characteristic of fatal shock.

During the transition from the initial vasoexcitor to the vasodepressor phase, it is of interest that the blood samples show neutral properties in the rat meso-appendix test. This indicates either that there is a disappearance of the vaso-excitor prior to the development of vasodepressor material, or that the progressive accumulation of vasodepressor material counterbalances and, later, overwhelms the vasoexcitor material already present. Evidence for the latter assumption has been found in experiments, shortly to be published, in which the chemical fractionation of neutral blood samples, taken during the transitional period, shows that both vasoexcitor and vasodepressor principles are present, but in counterbalancing amounts.

SUMMARY

A variety of animals subjected to acute and graded hemorrhage, hind-limb tourniquet, trauma in the Noble-Collip drum and leg-pounding, develop blood-borne, vasotropic substances. The substances were detected by injecting samples of blood (serum or heparinized plasma) into the tail-vein of normal rats and observing the effect on the intensity and duration of the reactivity of the terminal vessels in the exposed meso-appendix to the topical application of epinephrine. A vasoexcitor material (VEM) appears early after blood-loss. This is replaced by progressive, increasingly effective vasodepressor material (VDM) during the development of irreversibility of the shock syndrome. The appearance of a marked VDM effect was found to be correlated with the fatal outcome of the shock syndrome.

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THE EFFECT OF AMPHETAMINE SULFATE AND SOME BARBITURATES ON THE FATIGUE PRODUCED BY PROLONGED WAKEFULNESS[1]

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There are many reports of studies designed to investigate methods of alleviating the effects of prolonged or intense muscular activity but relatively few have appeared on the fatigue due to deprivation of sleep. Unlike intense muscular activity, prolonged wakefulness results principally in psychological disturbances and, with the exception of changes occurring in the electrical activity of the brain (1), there is little indication that insomnia, even up to 200 hours' duration, produces any significant physiological or biochemical alterations (2, 3, 4, 5). Certain psychomotor changes occur that are characterized chiefly by a somewhat slower reaction time and a decrease in ability to maintain sustained effort and performance. In general, the lack of definitive changes in visceral activities make it appear that deprivation of sleep results in a "fatigue" that is confined to the higher centers of the central nervous system.

It was of interest, therefore, to study the effects of some central nervous system stimulants and depressants on the fatigue produced during experimental insomnia. These interests were enhanced by problems arising out of the exigencies of the war. For instance, certain barbiturates were components of motion sickness remedies being considered for general use in the armed forces. Hence, it was desirable to obtain information on the effects of these drugs when given to men under such conditions of stress.

METHODS. The subjects were volunteers from Army, Marine Corps or Civilian Public Service Camps, and they ranged from 17 to 35 years in age. Prior to each experiment, the men accepted were apprised of the nature and purpose of the study (except those details which might influence the results, such as the type of medication, etc., to be employed). They were assured that they were free to drop out from the experiment at any time they wished, and it was made clear that should they do so it would not result in any reflection on their military record. During the experiment, when a man expressed a desire to quit no effort was made to induce him to continue. About 20 per cent of those who started failed to complete the test or were dropped for other reasons given below.

Twenty experiments were carried out between 1942 and 1945; three were of 24 hours' duration in which 63 men finished, five were 48 hours long in which 81 finished, four were 60 to 72 hour tests with 169 men finishing, and eight studies were of 112 hours' duration in which 275 of 350 volunteers finished.

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The program of activity, particularly in the 112 hour experiments, was, of necessity, heavy since some form of continuous physical activity was required to keep the men awake after the 2nd day. The "basic" activity of all the experiments was the routine training program peculiar to parachutists and other similarly specialized troops. In addition to this work program, constant physical activity was kept up by means of sports (basketball, baseball, swimming), walking, and special forms of military and tactical problems. In a few experiments an additional heavy work program was carried out by having the men make two night marches during the first two evenings, totalling from 35 to 65 miles, depending upon the condition and nature of the groups.

Short, carefully supervised rest periods, in addition to the rest at meal times, were allowed at regular intervals. At such times, the men were permitted to sit, but not to lie down or close their eyes since, after the second day, a few seconds in a prone position resulted in their quickly falling into a deep sleep.

The daily program for the shorter experiments was essentially the same.

Except in a few experiments reported elsewhere (4), the men subsisted on the regular camp mess, plus an additional meal at midnight, the caloric intake being estimated at about 6,000 calories per day.

In every experiment one sub-group received only placebos throughout and served as the control. Also, any time medication was given to one group all the others received a placebo of identical size and color.

In the 24 to 48 hour experiments, amphetamine sulfate (benzedrine sulfate) was given in a single dose of 10 mgm., either at the 12th, 18th, 24th or 36th hour. In the 72 hour tests, 10 mgm. benzedrine were given commencing either on the 36th or 48th hour and the dosage repeated every 8 to 12 hours. In the 112 hour experiments, 5 to 10 mgm. of benzedrine sulfate were administered beginning the 48th hour and the dose repeated every 8 to 12 hours, the final dosage being given at 6:00 p.m. of the last day. These time intervals were selected for the administration of benzedrine sulfate since it was considered the most practical and easily adaptable procedure for giving the drug in the field and under combat conditions.

Two different barbiturates were studied during the 112 hour experiments: sodium-iso-amylethyl-barbituric acid (amytal) and ethyl-β-methyl-allyl thiobarbituric acid ("V-12"). These were administered every 12 hours during the first 48 hours commencing at 6:00 a.m. of the first day and the last dose was given at 6:00 p.m. of the second day. The total amount administered was either 4 grains for amytal or 10 grains for V-12.

Most of the testing procedures used are well-known and need little description. These included reaction time, both simple and multiple choice, test for static ataxia, steadiness tests (including steering), tests for flicker fusion frequency, for auditory acuity, vibration sense (tuning fork sensitivity (6)), dark adaptation, vision tests for vergence, fusion, lateral balance and depth perception, target identification, tapping tests, tests for ability to estimate the passage of short periods of time (90 sec.), test for marksmanship, cancellation tests, digits span and digits reversed, group Rorschach examination, as well as routine physi-

cal examinations (at least once daily), which included pulse, blood pressure, temperature and neurological examinations.

The testing period started at sundown each day (usually 1 to 2 hrs. after the last medication) and lasted from 3 to 4 hours. A representative battery of the above enumerated tests were employed, large enough in number (generally 12 to 15) to ensure continuous activity of all the men during the testing period. As a rule, control values of the tests used were obtained over a 3 day period prior to the experiment. This was not always practicable, particularly when there were large numbers of subjects involved in an experiment. In such instances, only one day was allotted for the accumulation of these control values.

RESULTS. *Placebo controls.*[3] Since few of the tests showed significant changes from the control values, it is considered unnecessary to reproduce in full the findings on all the tests employed. Typical samples of the results of some of the procedures used are given in table 1. Nor will it be necessary to consider separately the 112 hour experiments in which varying dosages of benzedrine sulfate were used or extra heavy work carried out, for under the conditions of these studies, no discernible differences were found in the results of those individual experiments.

In general, where a psychomotor test was of relatively short duration and did not require a prolonged period of attention from the subject, no significant changes in performance occurred, even after 110 hours of wakefulness. If a procedure was prolonged by requiring a greater number of trials, or made unusually boresome (for example, 100 reaction time trials instead of 25), differences in performance became noticeable beginning about the 60th hour of sleeplessness (table 1A and B). Similarly, marksmanship based on the scores obtained with 10 to 15 rounds of ammunition was little affected by loss of sleep. When the Marine standard marksmanship test of 68 rounds was used, a deleterious effect was noticeable after 2 nights of sleeplessness (table 1C).

Many procedures suggested as indicators of fatigue of the central nervous system, when used under the conditions of these experiments, showed no changes even after 110 hours of insomnia. Flicker fusion frequency tests (7) and tuning fork vibration sensitivity tests (6) exhibited no significant differences from the control values (tables 1D and 1E). Static ataxia increased somewhat as sleeplessness progressed, but not significantly so (table 1F). No alterations from the normal were found in tests for auditory acuity (8), estimation of passage of time (90 sec.), digits span and digits reversed, and Rorschach during 112 hours of sleeplessness. In some tests, learning continued during the experimental period and by comparing the values found during this period with those found prior to the experiment and after the first night of sleep there was a slight indication that this process was interfered with. However, the differences, again, were not statistically reliable.

[3] Benzedrine sulfate and placebos were supplied by the Smith, Klein and French Laboratories, amytal and placebos by Eli Lilly and Company and V-12 and placebos by the Abbott Laboratories. We wish to express our appreciation of the generous aid given by these firms.

TABLE 1

GROUP	NUMBER OF MEN	CONTROL	DAYS OF INSOMNIA 1	2	3	4	5	POST SLEEP
A. Multiple choice reaction time (25 trials) duration of test—2 minutes								
Placebo	114	0.69	0.66	0.66	0.63	0.61	0.62	0.59
Benzedrine	66	0.70	0.67	0.62	*0.62*	*0.64*	*0.65*	0.59
V-12	47	0.68	*0.65*	*0.63*	0.61	0.62	0.62	0.55
Control (normal)	8	0.68	0.66	0.64	0.62	0.60	0.59	0.60
B. Multiple choice reaction time (100 trials) duration of test—10 minutes								
Placebo	22	0.70	0.66	0.68	0.69	0.67	0.70	0.62
Benzedrine	20	0.68	0.67	0.66	*0.62*[1]	*0.65*	*0.69*	0.60
Amytal	6	0.68	*0.69*	*0.71*	0.68	0.67	0.69	0.59
Control (normal)	8	0.71	0.68	0.66	0.61	0.60	0.59	0.62
C. Marksmanship (68 rounds possible score = 340) duration of test—2.5 hrs.								
Placebo	23	278			262 (−16)			
Benzedrine	22	279			*281* (+2)[2]			
V-12	22	284			273 (−11)			
D. Flicker fusion frequency								
Placebo	6	45.8[3]	45.3	46.4	45.9	45.1	45.6	45.1
Amytal	6	46.0[3]	*45.5*	*46.4*	45.3	45.4	45.6	46.0
E. Vibration sense (in seconds)								
Placebo	22	27.9	28.3	29.8	29.5	28.2	30.6	25.6
Benzedrine	21	30.0	32.5	31.8	*32.1*	*32.4*	*32.6*	25.4
V-12	22	26.7	*28.3*	*28.5*	29.3	27.1	30.2	23.7
F. Body sway, eyes closed (cm. in 30 sec.)								
Placebo	22	8.0	9.3	8.1	9.2	8.2	8.5	7.6
Benzedrine	21	7.8	8.3	10.1	*7.9*	*8.9*	*8.6*	7.0
V-12	22	8.7	*10.3*[4]	*11.8*[5]	11.1[6]	9.7	9.6	8.0
G. Ability to stay awake (see text)								
Placebo	22			9	12	13	5[7]	
Benzedrine	21			11	*5*	*8*	*6*[7]	
Barbiturates	22			*11*	10	10	6[7]	
H. Frequency of men dropping out								
Placebo		181[8]	17	18	6	4		136[9]
Benzedrine		113[8]	14	11	*2*			86[9]
Barbiturate		57[8]	*1*	*2*	1			53[9]

Italicized figures indicate that the medication represented by the group was given that day. On all other days placebos were given. By comparison of placebo with medication using individual differences (1) p does not quite reach the 5 per cent level (2) p = 0.01 (3) average of results for 3 consecutive days prior to the test (4) p = 0.05 (5) p = 0.01 (6) p = 0.05 (7) incomplete data, only up to 9:30 p.m. (8) number of men starting (9) number of men finishing.

No significant changes were found in the urinary excretion of 17-ketosteroids and creatinine, level of sugar and adrenaline-like substances in the blood, blood pressure, pulse, body weight and reflexes. The results of these latter studies are reported in detail elsewhere (4, 5).

The degree of difficulty experienced by the men in staying awake at various times was determined both by questioning and by keeping records of the number of times a man had to be awakened during the course of a day, as well as by grading his state of alertness or wakefulness during the testing periods (by his response to routine questions or commands). During the first night and second day no unusual trouble was encountered by any of the men. The first real difficulty was experienced on the second night. Thereafter the men found it harder to stay awake at night or in darkened rooms than during the day or in well-lit rooms. Despite the fact that most of the subjects maintained that they could "go on indefinitely" after the third night and felt they experienced much less trouble in staying awake after that time, more objective assessment belied those claims. In table 1G are presented data on the state of wakefulness based on the number of times a man had to be wakened during the regular evening rest periods.

The effect of benzedrine. In experiments up to 48 hours' duration, using modified psychomotor tests, no significant differences were found between the placebo groups and the groups receiving 10 mgm. of benzedrine sulfate at either 12, 18 or 24 hours. In the 72 to 112 hour experiments, where benzedrine sulfate was first administered after 36 to 48 hours of wakefulness and the dose repeated at intervals of 8 to 12 hours, the deterioration in performance occurring around the 60th hour was prevented. Such groups exhibited better marksmanship than the placebo groups (table 1C). Also, somewhat better scores were found in reaction time (table 1B), steadiness, cancellation tests and body sway (table 1F). A marked effect of benzedrine sulfate administered under these latter conditions was the decreased difficulty in remaining awake. This action was most noticeable during the third night. Maintaining the same dosage throughout the fourth and fifth days did not have the same beneficial effect either on performance or ability to stay awake as it had had on the third night.

The effect of barbiturates. Amytal and V-12 in the doses employed produced variable effects on performance during the first 48 hours of a 112 hour sleepless period. Body sway (table 1F), cancellation and steadiness were somewhat poorer than the placebo controls while flicker fusion frequency (table 1D) and reaction time (table 1B) remained unaffected. No unusual difficulty was encountered by these men in staying awake during the first night and second day, and the trouble experienced during the second night did not appear any greater than for the placebo controls. Upon stopping the administration of these drugs (on the morning of the third day), the performance of these groups was similar to that found for the placebo control groups.

Other findings. The effect of benzedrine sulfate on mood and behavior has been subject to much study (9). Unfortunately, in these experiments, motivation was a strong factor and the general euphoria present in all subjects, partic-

ularly after the 3rd day, probably contributed to masking the effect of the drugs on any changes in mood or attitude. In none of the experiments were the observers or subjects told the nature of the medication being tested. On the basis of outward appearance or behavior of the subjects it was impossible to accurately judge what type of medication had been given them. The only exception to this was on the 3rd day and night, for then those men who were receiving benzedrine sulfate required less prodding than the other groups to prevent them from falling asleep. After the 3rd day, the subjects themselves were poor judges as to the type of pill they had received.

Although in these experiments it was difficult to detect any perceptible differences in mood or attitude as a result of any medication, the number of men dropping out of an experiment may be taken as an indication of the mental attitude. During the first 2 days of the 112 hour experiments both the "placebo" and "benzedrine" groups were receiving dummy pills and only the "barbiturate" groups were getting active medication. During this period 20 per cent of the men receiving placebos dropped out while only 5 per cent of the men getting barbiturates quit. During the 3rd and 4th days 6 per cent of the placebo group dropped out while less than 2 per cent of the benzedrine sulfate group (now actually receiving the drug) quit (table 1H).

The number of men volunteering for a disagreeable task may also be taken as another indication of mood or mental attitude. On the 3rd night of one experiment involving 35 subjects, half of whom had been given benzedrine sulfate, the men were asked individually, and out of hearing of any other person, if they would volunteer to break a forced march record which had been set a week previously (35 miles with full pack in less than 10 hrs.). They had already completed two night marches totalling 65 miles, and a third night march would have been out of the question as all had sore feet and blisters. Nine of the 17 men who had received benzedrine sulfate volunteered, while only 2 of the group receiving placebos offered to make the try.

Alterations in personality and behavior as a result of experimental insomnia have been reported by others (2, 3). These psychological disturbances first became apparent on the second night and, in most of the subjects, were mild. They were characterized by increased irritability, particularly during inactivity, loss of memory, a tendency toward what may be termed hallucinations or illusions, inattention, apathy and irrelevant laughter and conversation. The extent of these disturbances was dependent somewhat upon the size of the experimental group, but more so on their activity. All these symptoms disappeared after sleep. One experiment in which an attempt was made to have the men carry on only sedentary occupations had to be discontinued on the third day due to the extreme irritability which developed in the men and a resultant lack of co-operation on their part.

In a few cases the psychological changes which occurred were not so mild. These developed during the first 48 hours of sleeplessness and while they were still receiving placebos. The first such instance occurred just before dawn of the third day during a 112 hour experiment involving 35 men. This subject be-

came extremely aggressive, developed delusions of grandeur (imagined himself on secret missions for the President, etc.), started unprovoked fights, became unmanageable, and had to be forcibly restrained. He was put to bed and slept through the entire day. On awakening, he had no recollection of the events of the previous night. An examination of his record disclosed no indication of any psychotic tendencies during his training period; he was well liked, and considered quiet and reserved by his friends.

Six other such cases occurred during the course of these studies: two were characterized by extreme irritability and aggressiveness requiring restraint; four cases by persistent auditory and visual hallucinations of such a nature that it was deemed desirable to have the men drop out. As in the case described above, none of these six men had a previous history of psychotic tendencies and, following sleep, all symptoms and all recollection of the preceding events disappeared.

Upon the conclusion of each experiment, the men were permitted to sleep as long as they desired. Following 112 hours of wakefulness, the length of such sleep varied from 8 to 14 hours. Except for a slight sluggishness lasting about an hour or two, they appeared, for the most part, completely refreshed. However, in the early studies one condition that was found to interfere with this recovery was the position of the men during sleep following the experiment. Upon going to bed they would fall asleep instantly in the first position assumed and would rarely change it during the night. This resulted in some complaints of numbness and temporary lameness of the arms and legs the next day. To prevent this, in the later experiments, the men were ordered to lie flat on their backs with their arms along their sides or folded across their chests to avoid positions that might interfere with the circulation in their limbs. With these precautions, such complaints did not occur in subsequent experiments. Tests on the morning after such sleep showed no changes from the pre-experimental control values other than those due to learning.

Discussion. In order to keep men awake for prolonged periods some form of continuous muscular activity is necessary. This condition, together with the fact that the experimental subjects must maintain for an unusually long time the normal upright position of the body, as well as be denied the muscular relaxation and diminution of tonus that is a concomitant of sleep would lead one to believe that muscular fatigue is a contributing factor to the effects of prolonged wakefulness. Although measurements of work were not made in these studies, casual observations indicated that the work capacity of the men at any stage was independent of the loss of sleep but related to the amount of time that had elapsed from a previous period of heavy work and, more important, to the motivation. For instance, in those experiments where an unusually heavy work program was given during the first two nights (two long marches), the men would be rebellious about going on a 4 or 5 mile walk on the 3rd, 4th or 5th day, asserting they were exhausted. But they would readily participate in a fast game of basketball, volley ball or water polo at any time, and would play for an hour or more in a manner indistinguishable from that of fresh, rested men. Undoubtedly,

a greater degree of muscular "fatigue" was present in the men during bouts of sleeplessness than under normal conditions; nevertheless such observations tend to show that it is not so severe as to be an important factor in these studies. It appears, then, that although exhaustion and extreme sleepiness always accompany extreme muscular weakness, prolonged wakefulness is not necessarily accompanied by great muscular fatigue.

What, then, is the cause of the "deterioration" in performance? As pointed out above, it was difficult to detect deterioration in performance. Only with a test that requires prolonged attention, which is unduly boresome, or is given under conditions that are conducive to falling asleep (eyes closed or in darkened rooms) will "deterioration" become evident. Examination of the individual records, where such deterioration occurred, indicated that it was not due to failure in the particular skill, per se, but to a momentary dozing off or diminution of attentiveness and ability to concentrate on the part of the sleepy subject. As further evidence, this drop in the performance of the men was closely correlated with the increasing sleepiness or difficulty in remaining awake. That the deterioration was not more apparent on the 2nd night was probably due to the fact that the testing period was held too early on that evening.

It could be expected, then, that any procedure which improved the ability of the men to remain awake, and hence increased their attentiveness under these circumstances would prevent the falling off in performance. Under certain conditions benzedrine sulfate had that effect. If it was administered in any experiment before 36 hours, it had no useful purpose for up to that time little difficulty in remaining awake was experienced and no deterioration in performance was found to occur. If, on the other hand, its administration was delayed until the time that greater difficulty in remaining awake was encountered, it improved the ability of the men to stay awake and, as a consequence of this action, the deterioration in performance of the men was prevented.

However, if the same dosage was repeated daily during the 112 hours its antihypnotic action diminished and, as a result, it had no effect on performance.

A possible explanation of the mechanism for these effects is suggested by other studies. It is known that increased attention or mental effort is accompanied by an increase in the rate of the electrical activity of the brain (10). It was shown (1) that the mental effort required of a subject to remain awake during experimental insomnia resulted in an increase in the rate of the potential changes in the brain far above the level found during the control days. Furthermore, if an additional effort, such as working a multiplication problem, was exacted of a sleepy subject the results pointed to the reduced capacity of such a fatigued brain to further increase the rate of its electrical activity in response to the additional stimuli (1). One of the actions of benzedrine sulfate is to increase the rate of the electrical activity of the brain (11), and it is suggested that by this action it contributes an important condition required for remaining awake during experimental insomnia.

Ivy (12, 13) and Dill (14) and their co-workers have noted an effect of benzedrine sulfate and other stimulants on the amount or character of the work output

only under those situations where the testing procedures were made unusually long or where the administration of the drugs was accompanied by a subjective feeling of relief from fatigue. It would appear that the above interpretation might also explain the action of such stimulants under conditions where the subject had had adequate sleep but the procedure produced a fatigue of the central nervous system.

The most outstanding findings as a result of loss of sleep are the psychological disturbances. For the most part, these became evident beginning on about the 3rd day. In general, this confirmed, on a more extensive scale, the results reported by others (2, 3). In addition, a few cases occurred which were not mild and, in fact, resembled symptoms of acute schizophrenia. That such incidents were observed for the first time in these experiments is no doubt due to the fact that a larger number of men were used and hence a more representative sample of their particular age group was obtained. Although the cases shown here fully recovered after the equivalent of a night's sleep, one cannot help but feel that the mechanism producing the psychological symptoms, such as apathy, bursts of unreasonable laughter, autistic expressions (which were apparently due to dreams occurring in the semi-sleep periods), and the tendency towards hallucinations might be related to that causing the permanent changes in certain psychotic disorders.

SUMMARY

From the results reported here and in previous publications (1, 4, 5, 8), it was found that:

1. With the exception of the effect on the electrical activity of the brain, no other significant physiological or biochemical changes were observed during 112 hours of experimental insomnia, and the indications are that the fatigue is confined to the higher centers of the central nervous system.
2. Performance based on tests of a wide variety of psychomotor skills shows little change as long as the tests are of short duration. Modifying a procedure to require a sustained effort on the part of the subject results in poorer performance, first becoming evident after about 60 hours of sleeplessness. The inability to sustain effort is related to the increased difficulty of remaining awake and not to any muscular weakness.
3. Benzedrine sulfate prevents such deterioration in performance when it is first administered between the 36th and 48th hour. A possible explanation for the mechanism of action of this drug under these conditions is offered.
4. Barbiturates in the dosages employed have only a slight effect on performance or the ability of the men to stay awake during such a vigil.
5. The chief disturbances produced by sleeplessness are psychological. In the great majority of the subjects these changes are mild and transitory, disappearing following sleep. The possible relationship between the mechanism producing these symptoms and the more permanent changes in certain psychotic disorders is discussed.

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BLOOD SUGAR LEVELS AND THE BEHAVIOR PATTERN OF YOUNG HEALTHY ADULTS SEVERAL HOURS AFTER THE INGESTION OF LARGE AMOUNTS OF SUCROSE[1]

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In a previous study (1) there could be found no evidence of hypoglycemia or of the so-called hypoglycemic syndrome after a breakfast containing approximately 600 calories, 81 per cent of which was derived from carbohydrate. Similar observations were made on a group of swimmers several hours after a heavy meal in which carbohydrate provided 76 per cent of the total calories. From these studies it was concluded that the hypoglycemic syndrome which can be relieved by restriction of the carbohydrate intake is a pathological condition and not a normal physiological response to a high carbohydrate meal.

In the same volume of the Journal in which our paper appeared there was another (2) in which entirely different results from ours were reported. The ingestion of 70 to 150 grams of dextrose was found to induce the development of "hypoglycemi's" reactions in 5 out of 6 subjects approximately 50 per cent of the total number of times tested. "These hypoglycemic episodes usually began within 3 to 5 hours after ingestion of the sugar and occurred with approximately equal frequency at either ground level or altitude. The seizures were characterized by subjective sensations of nervousness, impending danger, paresthesia, weakness and hunger, and objective manifestations of pallor, sweating, tremulousness, coldness of the extremities, and a tendency in some toward a drop in oral temperature."

In view of the advisability of routine carbohydrate feeding to flight personnel suggested by the experimental evidence that a high carbohydrate intake leads to a gain in altitude tolerance (3, 4, 5), it becomes of practical importance to establish unequivocally the existence or non-existence of a relationship between a high carbohydrate intake and a subsequent hypoglycemic syndrome. It is obvious that the "occurrence of such reactions as weakness, intense hunger, and tremor, either mild or severe, may lead to disastrous results in flight and would be especially dangerous should they develop at or about the time of landing" (2).

The present study was undertaken to determine whether the hypoclycemic syndrome similar to that described by D'Angelo (2) after dextrose administration would be induced by the more common article of food, sucrose.

Procedure. Forty-four medical students, three of whom were women, served as volunteer subjects. In order to avoid the possibility of suggestion playing any part in their subjective state, they were told that the purpose of

[1] The expense of this investigation was defrayed by a grant-in-aid from the Sugar Research Foundation.

the experiment was to obtain data on the sucrose tolerance curve. Care was taken to avoid any reference to the hypoglycemic syndrome.

On the day of the experiment the subjects came to the laboratory in the fasting state. Two basal blood samples were taken: arterial by finger puncture and venous by venepuncture. The subjects then drank a lemon-flavored sucrose solution containing 1.5 grams of the sugar per kilo body weight in 400 cc. water. For the remainder of the morning they continued in their usual activities reporting to the laboratory at hourly intervals for the next four hours for the drawing of blood samples. No other food was taken until the mid-day meal. Objective estimates were recorded by four members of the research staff on the physical and behavioral reactions of the subjects. In the course

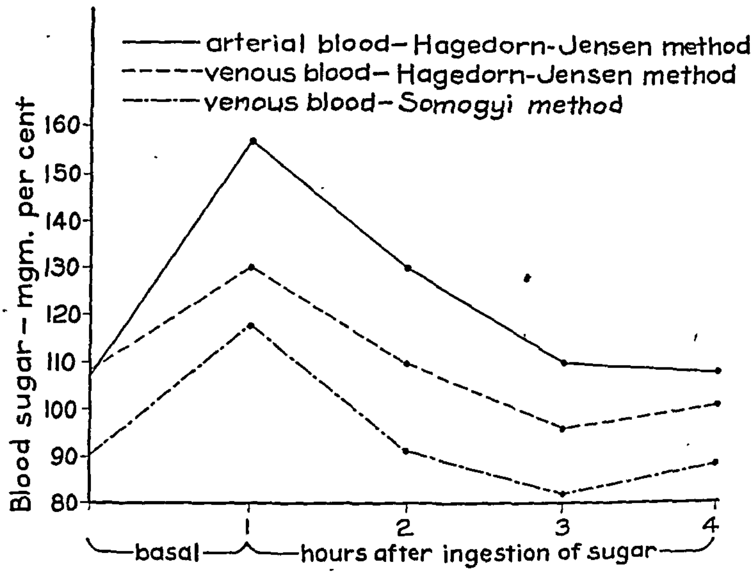

Fig. 1. Sugar concentrations in arterial and venous blood following the ingestion of 1.5 grams sucrose per kilo body weight in 400 cc. water.

of a general conversation with each subject at the conclusion of the experiment comments were elicited on how he felt. Again care was exercised to avoid suggestion.

The arterial blood was analyzed for sugar by the Hagedorn-Jensen procedure and venous blood by the Shaffer-Hartman-Somogyi method (6). In view of our previous experience that the Hagedorn-Jensen procedure in our laboratory yields values approximately 15 mgm. per cent higher than the Somogyi method it was first thought it would be satisfactory to apply a correction factor for equating the arterial and venous blood sugar concentrations. Later when the experiment was in progress it was decided to analyze the venous blood also by the Hagedorn-Jensen method in order to obtain a more accurate comparison between the arterial and venous blood sugar values. Venous samples obtained from 33 subjects were analyzed by both procedures.

Results. The average concentrations of sugar in arterial and venous blood throughout the experiment are presented in graphic form in figure 1. The

curves of venous blood sugar have the contour of those usually obtained in normal individuals on the glucose tolerance test. The concentrations which rose appreciably one hour after ingestion returned to the basal within two hours and dipped below this level the third hour. The concentration in arterial blood which was at the same level as in venous blood in the fasting state rose to an appreciably higher level in the first hour and returned more slowly to the basal than is the case with venous blood. Unlike the latter it did not fall below the basal.

Analysis of the individual data revealed that by the Somogyi method, (which gave lower values than the Hagedorn-Jensen procedure) only 9 out of 44 subjects had a blood sugar concentration below 80, 6 below 70, and 1 below 60 in the third hour after the ingestion of sugar. From these results it may be concluded that hypoglycemia, in the generally accepted sense of the term, is not a usual response to the ingestion of a large amount of sucrose.

TABLE 1

*Blood sugar concentrations after ingestion of glucose and of sucrose (1.5 grams/kilo in 400 cc. water)**

SUGAR INGESTED	BASAL	MINUTES AFTER INGESTION				
		2	5	10	15	30
	Blood sugar—mgm.%					
Glucose	95	99	108	125	134	155
Sucrose	95	100	107	122	134	148

* Each value is an average of 26 experiments.

The observations recorded by the objective observers were uniformly in agreement. In only two of the subjects were there manifest any symptoms or physical signs that bore a resemblance to the hypoglycemic syndrome. Both these subjects (one man and one woman) were somewhat nervous. Each complained of a sense of faintness. Perspiration was observed on the forehead and palms of the hands. However, it is important to note that in both these individuals these signs were present before sugar was taken. It is a reasonable assumption that fear over being stuck with a needle was responsible for the reaction. To all outward appearances the physical condition and behavioral pattern of all the other subjects were no different from those which had been observed in our previous frequent contacts with them.

Inspection of the subjective reports which were recorded after the interview showed that 31 of the 44 subjects felt "perfectly normal." Frequently the comment was volunteered by a subject that he was surprised to find he could get along just as well on the sugar as on his usual breakfast. Of the remaining 13 subjects, 8 stated that they felt hungry at 11:00 o'clock or at noon; two (as stated previously), appeared faint and nervous; one complained of headache at 12 o'clock; and two were somewhat nauseated from 10:00 o'clock on.

This discrepancy between our results with sucrose and those of D'Angelo

with dextrose suggested the possibility of a slower rate of absorption after sucrose ingestion. If such were the case a more rapid rise in the blood sugar concentration following the administration of dextrose might conceivably account for the difference in the results. This seemed improbable in view of the findings of Rabinowitch (7) that the sugar concentration of venous blood of diabetics showed an appreciable rise within 5 minutes after drinking a 5 per cent aqueous solution of sucrose containing 10 grams of sugar. However, it was considered advisable to obtain comparative determinations on the rise of arterial (finger tip) blood on normal subjects after ingestion of glucose and sucrose. One and five-tenths grams of these two sugars per kilo body weight was administered on different days to the same subjects. The sugar was dissolved in 400 cc. of water and two minutes allowed for drinking the entire amount. The averages of 26 experiments with each sugar on 9 subjects are presented in table 1.

From these observations it is apparent that the sugar concentration of the blood rises with equal rapidity after the ingestion of glucose and of sucrose. Obviously sucrose is inverted promptly in the gastro-intestinal tract.

The sugar was consumed in these experiments at 2:30 in the afternoon. No other food was eaten before the evening meal $3\frac{1}{2}$ to $4\frac{1}{2}$ hours later. In no instance did the subjects experience the symptoms of the hypoglycemic reaction.

CONCLUSIONS

A study of the blood sugar concentration and the behavioral response of 44 healthy young adults failed to reveal any evidence of the so-called hypoglycemic syndrome during a four hour period following the ingestion of 1.5 grams sucrose or of dextrose per kilo body weight.

The blood sugar concentration rose with equal rapidity after the ingestion of sucrose and of dextrose.

These experiments offer confirmatory evidence for the conclusion drawn from previous studies that the hypoglycemic syndrome is not the usual response of normal healthy adults to a high carbohydrate intake.

Correction in Figure one. The *first point only*, i. e. those on the ordinates, are the basal values. The sucrose was injected immediately after drawing the basal blood sample.

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RECORDING OF BLOOD PRESSURE FROM THE LEFT AURICLE AND THE PULMONARY VEINS IN HUMAN SUBJECTS WITH INTERAURICULAR SEPTAL DEFECT[1]

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Pressure tracings from the left auricle and the pulmonary veins have been obtained, using the cardiac catheterization technique (1, 2, 3, 4) in three young human subjects, 5 months, 2 years and 5 years of age, with an interauricular septal defect; and compared with pressure tracings in the right auricle. As far as can be ascertained such tracings have not been previously reported in man. The auricular septal defect was not associated in any of the cases with a demonstrable anomaly of the mitral or tricuspid valve. There was no evidence of cardiac decompensation at the time of study, and on the basis of the fluoroscopic findings there was no apparent dilation of the right or left auricle. The systolic blood pressure in the right ventricle was elevated in the 3 cases, presumably as a result of an increase in pulmonary flow; however, in one case a moderate degree of pulmonary stenosis could be demonstrated, the pulmonary artery systolic pressure (33 mm. Hg) being lower than the right ventricular systolic pressure (59 mm. Hg).

TECHNIQUE. A no. 6 French ureteral radio-opaque catheter was introduced into the venous system by way of the internal saphenous vein exposed in the femoral region. Under fluoroscopic control the tip of the catheter was placed successively in the right auricle, the left auricle and one of the pulmonary veins (fig. 1). Tracings were obtained, within a short time interval, from the various locations, using a Hamilton manometer and/or an electrical apparatus (5) for the pressure recordings. Electrocardiograms were taken simultaneously using the standard lead II. The time lag between electrical events recorded with the electrocardiograph, and mechanical events simultaneously induced and recorded, was found to be 0.01 second, for both types of manometers. During the entire procedure, a steady state of the respiration and circulation was maintained, the subjects being under avertin anesthesia.

RESULTS. In the top row of figure 2 appear pressure tracings taken with the Hamilton manometer a few minutes apart from the right auricle and from the left auricle of the child 5 months old. In the bottom row is illustrated a tracing recorded from the left auricle, of a child, age 2 years, using the electrical recorder. The similarity of both tracings of left auricular blood pressure is

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Fig. 1. X-ray of the chest in a case of a 5 month old child with the tip of the catheter located in the right auricle (left) and in a pulmonary vein (right). The catheter was introduced into the venous system through the right internal saphenous vein at the femoral region. Its tip is located in the right auricle (left x-ray picture), and then passed through an interauricular septal defect into the left auricle and a left pulmonary vein (right x-ray picture). Note that the catheter tip lies outside the heart shadow in the latter picture.

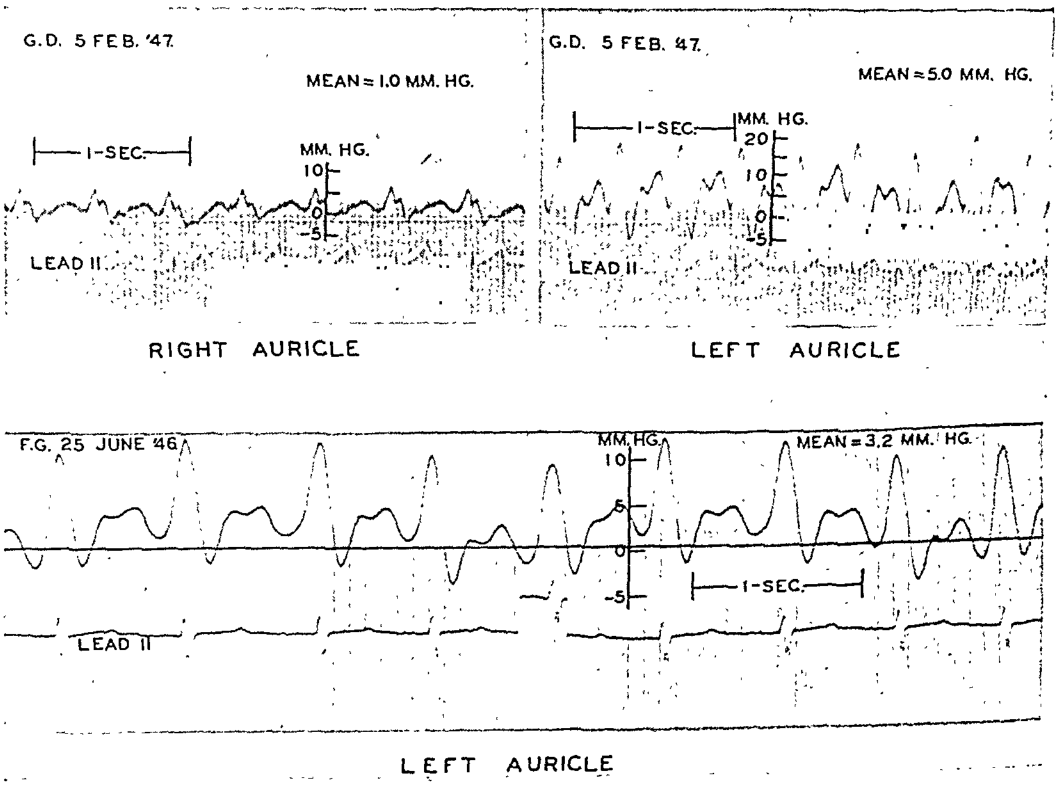

Fig. 2. Tracings of blood pressure taken from the right auricle (upper row—left) and the left auricle (upper row—right and lower row).

Both records in the upper row were taken within a few minutes interval, in a 5 month old child, under avertin anesthesia, through a number 6 catheter connected with a Hamilton manometer. The time lag in the recording system, including the catheter, between electrical and mechanical events was 0.01 second. There is no parallax between the electrocardiogram and the pressure tracings. Note the marked pulse rate variation and the inversion of the P wave on the left ECG tracing.

The record in the lower row was taken in a two and one half year old child through a number 6 catheter connected with an electrical recording system (see text).

striking with regard to the shape of the curve and the amplitude of the pressure variations during the cardiac cycle.

Tracings from the left auricle, however, differed greatly from blood pressure curves of the right auricle in the following characteristics: *a.* The pressure rise during the auricular systole was greater, and in 2 cases where the tracings could be satisfactorily compared, the pressure at the peak of auricular systolic wave in the left auricle averaged 13.1 mm. Hg as against 4.7 mm. Hg in the right. *b.* The slightly positive wave or notch corresponding to the onset of ventricular systole, clearly visible after the right auricular systolic wave, was not visible on the left auricular tracing. *c.* The pressure decrease in the auricle during the period corresponding to the descent of the base was much greater in the left than in the right auricular tracing. *d.* The pressure rise simultaneous with the ventricular ejection phase, and corresponding to the rapid phase of auricular filling, was much greater in the left auricle, averaging 11.7 mm. Hg as compared to 3.7 mm. Hg in the right, and its slope was much steeper.

TABLE 1

Mean pressure measurements in the right and left auricles and in the left pulmonary veins

CASE	AGE	RIGHT AURICLE	LEFT AURICLE	LEFT PULMONARY VEIN
		mm. Hg	*mm. Hg*	*mm. Hg*
G. D. no. 329	5 months	+1.0	+5.0	+7.0
F. G. no. 267	2 years	+1.6	+3.2	+5.0
C. G. no. 296	5 years	+1.5	+4.0	+6.5
Average		+1.4	+4.1	+6.2

e. Finally, the decrease in pressure corresponding to diastolic inflow was more marked in the left than in the right auricular tracing.

In table 1 are tabulated the mean pressures in the right and left auricles and pulmonary veins, obtained by planimetric integration of the area under the pressure curves and averaged for the 3 cases. The average mean pressure in the right auricle checks well with figures obtained in normal adults. The pressure gradient between the left mean auricular pressure and the right, respectively 4.1 mm. Hg and 1.4 mm. Hg is very significant.

Discussion. It has long been suspected by clinicians that the pulmonary blood flow is considerably increased in cases of interauricular septal defect. Presumably this large blood flow is due to shunting in variable amount of pulmonary venous blood from the left to the right auricle and thus adding to the returning systemic blood flow. The observations reported here give objective evidence of such a shunt. The mean pressure being higher in the left than in the right auricle, provides a pressure gradient which favors the shunting of blood through the septal defect in the expected direction. Since it is known, however, that in cases of interauricular septal defect the arterial oxygen saturation is sometimes lower than normal, especially during periods of increased

activity, shunting of blood from the right to the left auricle is also a distinct possibility. An analysis of instantaneous pressure differences between both auricles would be required to provide factual evidence of this additional shunt. Using a double lumen catheter (6) for simultaneous recording of pressures in both auricles would seem to be the best method to provide pressure records for such analyses. The size of the venous system in the young subjects studied precluded the use of this type of catheter. However, a reversal of interauricular blood flow, proceeding from right to left during a short phase of the cardiac cycle, may be assumed on the basis of the distinctly lower level of pressure attained in the left than in the right auricle during the period corresponding to the descent of the base.

In analyzing the factors contributing to differences in contour and pressures in tracings taken from each auricle the following should be emphasized: *a*, changes in hydrostatic levels affecting both differentially; *b*, transmission of pressures from one to the other through the abnormal communication; *c*, separate dynamic conditions prevailing in each.

Wiggers (8) has suggested that the left auricle might be more affected than the right by the up and down movements of the ventricular base. It seems unlikely that shifts in the hydrostatic level account alone for the large differences in form and pressure.

If for the sake of discussion, the presence of an abnormal communication in the interauricular septum is invoked to explain the higher peak of the left auricular systole, the following sequence of events may be postulated: (1) As the wave of excitation from the S-A node (impulse) has been shown to arrive 0.015 sec. sooner in the right auricle than in the left, the earlier effects of pressure rise in the right auricle would be transmitted through the defect; (2) these would result in an increase in volume of and initial tension in the left auricle before its systolic contraction starts; (3) the higher systolic peak would, therefore, be due to a summation of effects of increased initial tension and energy produced by the left auricular contraction. However, the very large pressure difference between the peaks of auricular systole in each chamber, averaging 8.4 mm. Hg would seem to argue against this explanation.

Indeed, the other individual characteristics observed in the left auricular tracings, i.e., the large pressure drop corresponding to the descent of the base, the steeper slope and more rapid rise of pressure during ventricular contraction, cannot be accounted for unless the notion of inherently distinct dynamic conditions in both chambers is introduced. There are anatomic differences between (a) the two auricles, the left auricular wall being thicker than the right; (b) their venous reservoir, the 4 pulmonary veins being shorter and their diameter smaller than the superior or inferior vena cava and (c) both ventricles, the muscular development of the left ventricle being much greater than the right. These anatomic differences suggest that the left auricle is less deformable than the right; that its venous reservoir has a smaller capacity, and finally that effects of muscular activity of the left ventricle upon volume and tension in the left auricle may be more pronounced than similar activity of the right

ventricle upon volume and tension in the right auricle. Such dynamic differences are compatible with the tracings reported. They would tend to explain, in addition, why the large pressure changes in the left auricle do not influence pressure conditions in the right auricle, as they are transmitted through the defect.

While pressure curves in the left auricle have been reported many times in dogs, (8) there is a lack of information concerning the amplitude of pressure variations. A casual reference is made in Starling's Physiology to a higher pressure in the left auricle than in the right. According to Wiggers (9) who has had considerable experience in recording left auricular pressures in dogs with open chests, most of these have not been measured as it is not easy to obtain auricular pressure records free from extra-cardiac impacts especially in the case of the left auricle. However, Hamilton, Woodbury and Vogt (10) have shown that pressure variations in the pulmonary veins of intact, unanesthetized dogs were of large amplitude during each cardiac cycle providing, therefore, some suggestive evidence that there may be differences in the dynamics of the two auricles of the dog.

SUMMARY

1. In 3 human subjects with an interauricular septal defect pressure tracings were obtained successively from the right auricle, the left auricle and a pulmonary vein.

2. The amplitude of pressure variations and the mean pressure in the left auricle was greater than in the right.

3. The importance of these findings in relation to the mechanism of blood shunt in interauricular septal defect is discussed.

4. It is suggested that in normal man dynamic conditions may not be identical in both auricles.

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AN ANALYSIS OF CHANGES IN THE CONTOUR OF THE FEMORAL ARTERIAL PULSE DURING HEMORRHAGIC SHOCK[1]

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While a variety of indices of the development of circulatory shock are now available for the experimentalist, the clinician must still rely almost exclusively upon determinations of blood pressure and palpation of the peripheral arterial pulse in order to follow the course of circulatory failure. It is a common observation that shortly after a moderate hemorrhage in which blood pressure is reasonably well maintained the peripheral pulse may become intensified and assume a bounding quality. If blood pressure subsequently falls toward terminal shock levels this pulse is gradually replaced by a thready pulse of small amplitude and rapid rate. An analysis of these pulse forms with the aid of optical recording methods would not only serve to clarify the cause of changes in the pulse in shock, but also might offer information as to alterations in the arterial system during the circulatory failure. The femoral arterial pulse of the dog is particularly well suited for such an experimental analysis because it represents the peripheral pulse whose genesis has been most thoroughly investigated.

Shock Technique. In order to determine whether the peripheral pulses during progressive circulatory failure of hemorrhagic shock show specific characteristics, it is necessary to compare them with pulse forms which are observed during benign hypodynamic states. Such comparisons may be made by using the step-wise hemorrhage method described by Lawson (12) in the bleeding and reinfusion procedure developed in this laboratory for production of standardized hemorrhagic shock (11, 19). Dogs were anesthetized with sodium barbital (ca 280 mgm./kgm.). Mean pressures were recorded by cannulating the right axillary artery. Pressure was reduced to 50 mm. Hg by repeated withdrawals of 2 cc. of blood per kgm. from a femoral artery every two minutes. In some experiments it was lowered temporarily to 30 mm. to make observations at this level and then sufficient blood was promptly reinfused to return the animal to the 50 mm. level. By recording optical pulses during the interval of stabilized blood pressure before each bleeding, control records were obtained of the pressure pulses at successively lower levels of simple hemorrhagic hypotension. The pressure was maintained at 50 mm. Hg for 90 minutes. It was then reduced to 30 mm. Hg and maintained there until either respiratory failure threatened or it proved necessary to reinfuse more than 10 cc./kgm. of the withdrawn blood in order to prevent decline of mean pressure below 30 mm.

[1] This investigation sponsored by a grant from the Commonwealth Fund.

All withdrawn blood, properly warmed and filtered, was then reinjected by a femoral vein. One of the two criteria for terminating the hypotensive period was usually encountered after 40 to 60 minutes at the 30 mm. level. Of a total of 22 dogs submitted to this procedure, 9 exhibited fulminant failure and death either at or shortly after the end of the 30 mm. period, 10 returned to a good blood pressure level after reinfusion but died in shock 45 to 300 minutes later, 1 showed a protracted failure, and 2 gave no indications of shock.

Technique for Recording Central and Femoral Pressure Pulses. Optical pulses were recorded as lateral pressures in order to exclude the necessity of considering the kinetic factors in the blood column in the interpretation of the pulse tracings. Lateral aortic pressures were recorded by passing a sound down the left carotid artery. Lateral femoral pressure was obtained by inserting a 15 gauge needle about 1.5 cm. in length into a small branch of the femoral artery until its tip came flush with and at right angles to the wall of the femoral artery. In some experiments a third pressure pulse was recorded by means of a 13 or 15 gauge needle inserted into the dorsalis pedis artery a few centimeters below the ankle joint.

The optical manometers were similar in design to those used by Green (6) except that a tense rubber membrane was used in preference to the glass membrane and the manometer was supported in a special clamp which provided accurate adjustments for the horizontal position of the light beam. The adequacy of the manometers was indicated by natural frequencies of 125 to 150 per second when connected with the aortic sound and frequencies of 80 to 110 when connected to the needles used for femoral and dorsalis pedis pulses.

The optical system represented somewhat of a departure from other methods currently in use (cf. 6). In order to take advantage of the sharp image obtainable with the slit lamp and still avoid parallax a simplified multiple slit lamp was employed. This consists of a 250 watt projection lamp shining through a metal plate in which are machined a series of vertical slits 1 mm. wide and 3 mm. apart. A large 25 diopter cylindrical lens with long axis vertical placed immediately behind the plate and a 4 diopter spherical lens in a draw-tube in front of the plate serve to flood the manometer bench with a whole series of slit beams. By slight lateral movement the mirror of any manometer may be brought into the center of one of these beams. The mirrors are 0.5 diopter silvered lenses which make possible the focusing of a sharp image of the slit at the camera placed 2 meters from the manometers. Adequate recordings are obtained with this arrangement at the recording speed of 120 mm. per second.

Any critical worker with sensitive optical recording equipment is familiar with the fact that calibration levels are prone to shift from time to time in spite of rigorous precautions to avoid this in the design and manipulation of the instruments. Therefore, after *each* recording the manometers were switched from the animal to a pressure system and a short recording made with the system at some standard calibration pressure in reference to fixed base-lines projected from stationary mirrors on the manometers. Pressures in all instances were standardized in reference to the hydrostatic level of the carotid artery.

General Characteristics of Central and Peripheral Pressure Pulses. The upper graph of figure 1 shows changes in mean arterial pressure during stepwise hemorrhage and during the maintained periods of 50 mm. and 30 mm. hypotension; the latter eventuating in death in this instance. Illustrative optical records of aortic and femoral pressure pulses obtained at times marked A–J are shown in sections of records below. The aortic pressure curves (A–G) show progressive reduction in pulse pressure and a slower elevation of the ascending limb. The diastolic limb shows a progressive decrease in gradient, and in segment F has become quite flat. These transformations are essentially similar to those which follow rapid massive bleeding, the only difference being that the characteristic development of a primary spike does not occur. This was also noted by Werle et al. (19).

The femoral pressure pulses in this series show a variety of changes which can be classified into four general types. The typical normal form shown in segment A of figure 1 may be termed type I. It is characterized by rolling primary and dicrotic waves with a pulse pressure 1.5 to 2.0 times that of the aortic pulse pressure. While aortic pulses in segments C and D do not change significantly in form and display a slight decrease in pulse pressure, the femoral pulse shows a more rapid rise and fall of the primary wave with a pulse pressure increasing to almost three times that of the central pulse pressure. There is also a tendency for the positive dicrotic wave to become more prominent. This form may be identified by designating it type II. In records E–G while the central pulse still shows relatively minor changes in contour the femoral pulse shows a progressive slowing in the rise and fall of the primary wave and a reduction in the dicrotic wave until in segment G the dicrotic wave virtually disappears. These pulses are verging towards a type III pulse in which, although the femoral pulse pressure is still significantly greater than the central pulse pressure, the primary wave is greatly broadened and the dicrotic waves are absent. A more striking form of this type III pulse will be observed below in the right hand recording for dog 5 in figure 6. Finally, in segments H–I the form of the central pulse is markedly changed to an abrupt rise and fall during systole without much change in numerical pulse pressure. Yet the femoral pulse becomes greatly broadened and for the first time the femoral pulse pressure becomes smaller than that in the aorta. These femoral pulses may be called type IV.

Physical Factors Affecting the Form and Amplitude of Femoral Pulses. It is well established that the peripheral pulse represents a fundamental pressure wave created in the aorta and transmitted toward the periphery which summates with waves reflected back from the periphery. According to Hamilton and Dow (9) the waves reflected from peripheral arterial beds below the thorax are projected back to the aorta almost simultaneously and by summation produce a large pressure oscillation or "standing wave" in the central compression chamber. This summated reflected wave usually rises just after the foot of the transmitted fundamental wave has reached the femoral artery. As a result, the two waves merge indistinguishably and raise the systolic peak pres-

sure in the femoral artery to a level which is considerably higher than systolic aortic pressure.

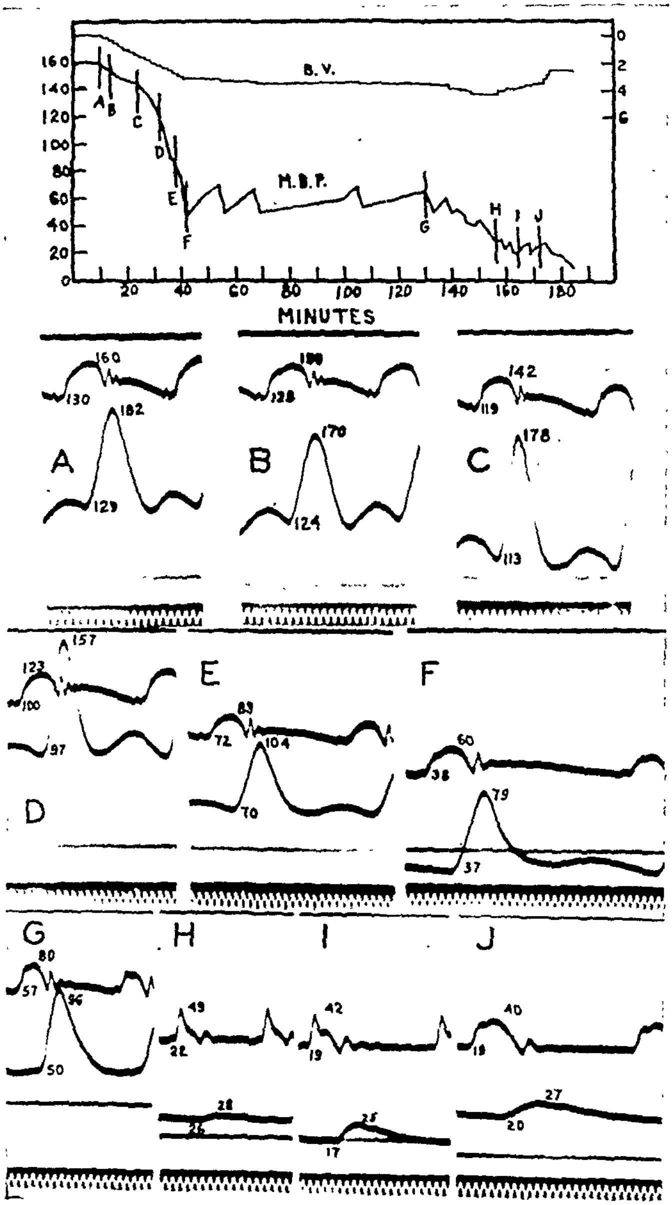

Fig. 1. Dog 4. Upper graph: Plot of bleeding volume (BV) expressed as per cent of body weight and mean blood pressure (MBP) in millimeters Hg for entire experiment. Pulse tracings shown below obtained at the points indicated. Upper tracing in each record: aortic pulse; lower tracing: lateral femoral pulse. Systolic and diastolic pressures indicated. Time signal in all recordings indicates 0.02 second for each double vibration.

The ways in which these summations can change are shown schematically in figure 2. The top row shows a typical aortic pulse as well as the transmitted

fundamental wave and the standing waves produced by reflections under normal conditions. For the sake of simplicity, the form of the transmitted wave is pictured as that of the central pulse with rapid oscillations damped out. The summation of the reflected waves is assumed to produce a simple sinusoidal

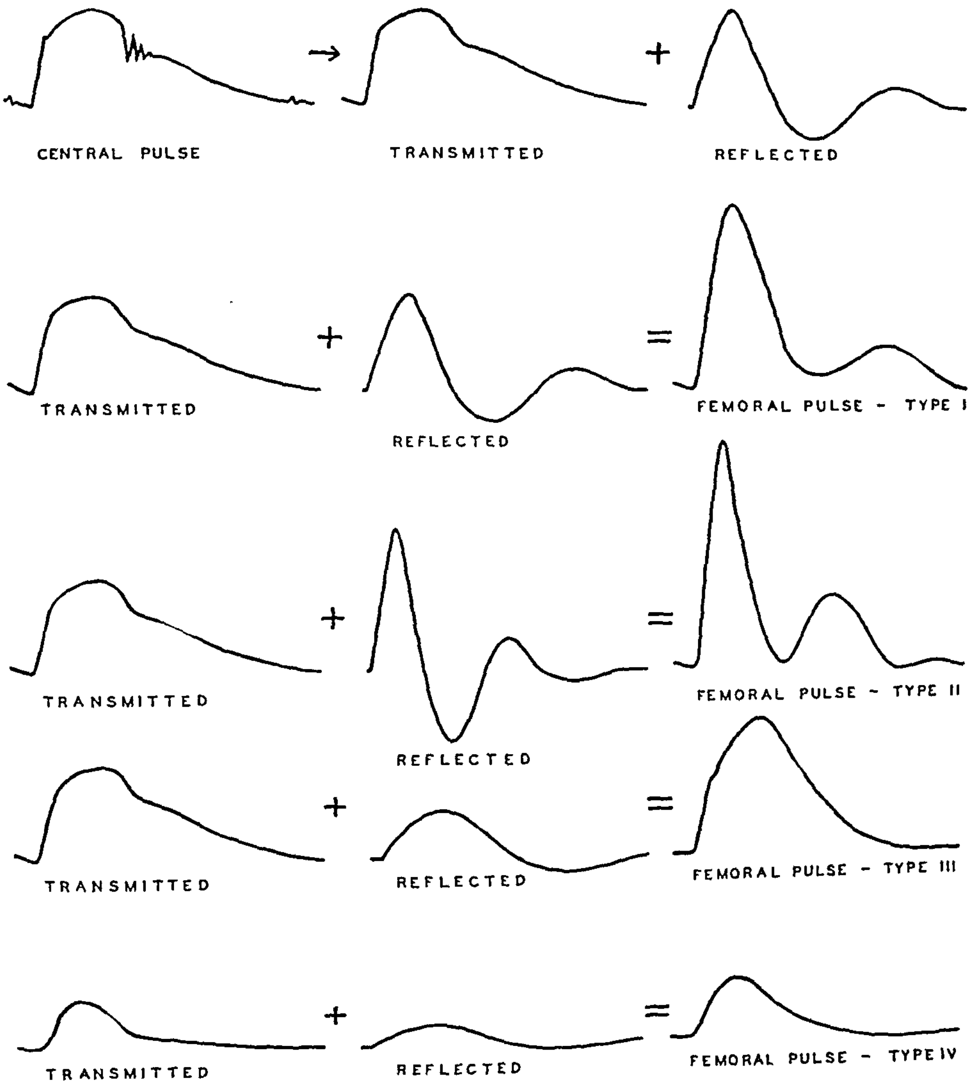

Fig. 2. Schematic synthesis of femoral pulse types as described in text

standing wave. Although neither of these assumptions are strictly correct they serve quite well for a first approximation. The second row in figure 2 illustrates the addition of the two components to form the typical normal femoral pulse of type I. The third row illustrates the changes that may be anticipated if for any reason the distensibility of the compression chamber decreases; the fourth row the changes that would similarly result if the distensibility increases. It will be observed that the resultant effects on femoral

pulse contour correspond to changes in the pulse form which have been identified above as types II and III. The cause of these changes is obvious. Whenever the distensibility of larger arteries decreases, i.e., when their walls become more tense, the period of the summated standing wave decreases and its amplitude increases. Since the speed at which both fundamental and reflected waves are transmitted will increase, the waves will summate earlier. Vice versa, when the distensibility increases the standing wave produced by the summated reflections will have a longer period and a decreased amplitude. Since the waves are transmitted more slowly, summation with the fundamental transmitted wave is delayed until it approaches its summit.

It is more difficult to resolve the factors which enter into the development of the type IV femoral pulse that is observed at very low blood pressures. Since peripheral pulse pressures are significantly less than aortic in this type, it is obvious that there must be a great damping of the central pulse in its transmission. If any reflected component is present under these conditions it must be of extremely low amplitude and frequency due to passive release of elastic tension in the arterial tree as a result of the great reduction in diastolic volume. The combination of these two factors would lead to a pulse contour whose components might be pictured as in the last row of figure 2.

While such a theoretical synthesis of pulse types does not in itself prove the mechanism of changes in pulse contour observed in the animal, it does offer a rational approach to the interpretation of pulse form. The algebraic subtraction of all curves to determine what changes occur in the form of the reflected wave system represents too arduous a task to be practical. It also would be subject to the criticism that the precise form of the transmitted wave is not definitely known. However, by measuring a few critical variables a fair estimate of changes in the properties of the arterial tree may be made. First, the elastic condition of the aorta may be assessed by measuring the transmission rate (TR) of the pulse wave to the femoral artery. When such measurements are made between two fixed points in the same animal one avoids the objections that have been raised against the use of pulse transmission rates for such a purpose (20). Secondly, since the peak of the femoral pulse represents the addition of a reflected wave to the transmitted wave, the difference between the femoral pulse pressure (FPP) and the central pulse pressure (CPP) should indicate the approximate magnitude of the summated reflected wave. When expressed as the ratio: FPP/CPP, values greater than 1.0 should be indicative of the addition of a significant reflected component in the femoral pulse. Finally, since the peak contour of the femoral pulse is primarily determined by the reflected components in pulse types I, II, and III, the time interval required for the pulse to rise from its foot to its peak (FP) should give an estimate of the speed of reflection and the period of the standing wave. Similar information should be obtained by measuring the rate at which the wave falls from the peak toward the dicrotic notch. The time required for the catacrotic limb to fall half way from its peak to the diastolic level ($\frac{1}{2}$ PF) was selected for this determination in order to avoid the dicrotic phase of the pulse. The

dicrotic waves themselves have been found to involve too many complicating features to submit to any rigorous quantitative analysis. That the dicrotic portions of the pulse waves do exhibit certain general changes in agreement with predictions will become evident in the further analysis of the original tracings.

To correct for passive changes in elasticity due to changes in the dynamic level of the system appropriate pressures had to be selected to which each set of measurements could be related. From a detailed study of the data as well as on the basis of certain theoretical considerations the following correlations appeared most significant: Transmission rate of the pulse wave (TR) was related to the diastolic pressure existing in the aorta at the *moment of initiation* of the pulse wave (3). The duration of the anacrotic limb of the femoral pulse (FP) was related to the diastolic pressure existing in the femoral artery at the onset of the pressure rise, and the catacrotic fall of the femoral pulse (½ PF) was

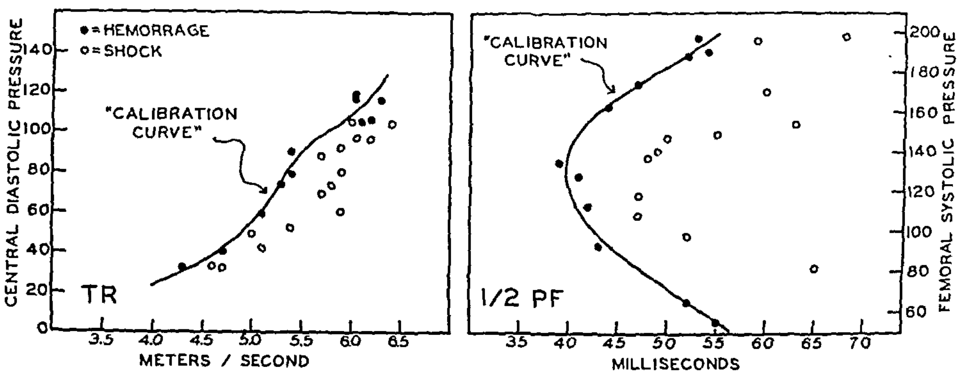

Fig. 3. Plot of raw data for transmission rate (TR) and duration of the first half of the catacrotic fall of the femoral pulse (½ PF) for dog 22, illustrating the calibration technique. Solid circles indicate values obtained during the hemorrhage, open circles indicate values obtained during the post-reinfusion shock.

related to the systolic pressure existing in the femoral artery at the moment the pulse started its descent.

Calibration Technique for Pulse Comparisons. When values for these respective variables were plotted respectively during the period of initial hemorrhage and post-reinfusion circulatory failure, certain general trends of the data became apparent. Figure 3 shows plots of such relationships of TR and ½ PF against their respective pressures. Values plotted in this manner failed to reveal trends during the initial period of hemorrhage which were in accord with any simple dynamic explanation based on the known volume-elasticity properties of the excised aorta of the dog. Pulse transmission rates consistently failed to exhibit an essentially linear trend as pressures lowered (10), tending rather to show an inflection in their course towards relatively more rapid transmission. The marked bowing of the ½ PF data early in hemorrhage towards a more rapid fall in pressure from the systolic peak indicates a transformation to a femoral pulse form of type II in this animal, in keeping with the similar

trend in dog 4 described above (fig. 1). These observations all point toward the occurrence of an active tensing of the central arterial system as hemorrhage is instituted. Since it is during this same period that compensatory vasoconstriction is known to occur it would appear that under these conditions, at least, the vasoconstrictor response involves a tensing of the central arterial vessels as well as a constriction of the arterioles. Such a parallelism between the response of the small vessels and the larger vessels has been assumed to occur generally by most investigators in the field (cf. review by Bazett (2)). As described above, after this initial shift towards type II, further bleeding leads to a more marked fall in blood pressure accompanied by a transformation of the femoral pulse towards type III. Vasomotor changes are not necessary to account for this latter shift, since it obviously represents the passive relaxation of the central arteries as the volume of blood distending them is decreased.

If post-reinfusion pulse contours are to be compared with these forms observed during hemorrhage, it is obvious that some allowance must be made for the compensatory alteration in pulse form as blood pressure lowers. This has been accomplished by regarding the values obtained during the hemorrhage period for each individual animal as the "control" response of this animal to a lowered blood pressure. "Calibration curves," indicated by the continuous lines in figure 3, were therefore constructed through these points and all data could then be expressed as per cent deviations from these curves at the corresponding pressure. When plotted in this manner as in figure 4 the calibration curve is represented by the dashed horizontal line at zero per cent deviation.

It should be emphasized that this method of analysis automatically corrects for both physical and physiological variables that are encountered in the dynamic changes resulting from a simple hemorrhagic fall in pressure. Since the total hemorrhagic period involved a progressive fall in blood pressure over a period of 40 minutes, it is assumed that all the normal compensatory reflexes to an acute fall in blood pressure had ample time to exert their influence on the calibration curves. To test the reliability of these calibration curves we made a second series of measurements of different pulses from the same recordings of the hemorrhage periods of dogs 13 and 22. The standard deviations of this series of twenty additional sets of measurements from the calibration values were $\pm$ 3.2 per cent for TR, $\pm$ 3.5 per cent for FP, and $\pm$ 3.8 per cent for $\frac{1}{2}$ PF. These deviations appear to represent little more than the error of measurement itself. Within the limits of accuracy with which the intervals could be measured, therefore, the calibration curves are a reliable description of the manner in which the respective variables were altered by the progressive lowering of the blood pressure during the period of hemorrhage.

Changes in Pulse Contour During the Hypotensive Periods. Illustrative examples of the pulse changes in an animal that failed to respond to reinfusion at the end of the hypotensive period have already been presented in figure 1 above. Of the ten animals which died in shock after exhibiting reasonably adequate responses on reinfusion of the withdrawn blood three have been selected as best representing the typical trends observed in the course of the

circulatory failure. The analysis of the records for two of these are presented in detail in figure 4. A series of original pulse recordings for the third of these animals is shown in figure 5.

The general trends during the hypotensive periods are best illustrated in the data for dog 13 in the upper part of figure 4. In most instances there is an acceleration in heart rate during this period coupled with an increase in the pulse transmission rate above the calibration value. The possibility that these transmission rates might be influenced by hematocrit changes has been excluded (1). This evidence of a tensing of the aorta at the time the heart accelerates is again indicative of a further compensatory response of the cardio-accelerator vasoconstrictor system. If our predictions outlined above in figure 2 are correct this tensing of the central arteries should be accompanied by a more rapid rise and fall of the peak of the femoral pulse. That such a change actually does occur during the 50 mm. period is indicated by a rise of both the FP and the $\frac{1}{2}$ PF intervals above the calibration level.

During the 30 mm. portion of the hypotensive period the changes are much more variable and dependent in part on the precise course of the particular animal considered. In general, however, this period was characterized by a tendency for all the variables to fall, the $\frac{1}{2}$ PF interval dropping far below the calibration level. It should be noted that at the same time the FPP/CPP ratio drops well below 1.0, indicating a pulse form of type IV. Hamilton (8) has interpreted the disappearance of a reflected component from the femoral pulse as being indicative of vasodilatation. In this case such an interpretation would be entirely unwarranted, since the change in the elastic tension of the arterial tree is quite obviously the passive result of the drastic reduction in blood volume. Indeed, in the face of this dominant source of laxity in the arterial system, it is quite impossible to draw any conclusions as to the vasomotor status of the animal from type IV pulses.

Changes in Pulse Contour During the Post-reinfusion Period of Circulatory Failure. Inspection of figure 4 reveals that immediately after reinfusion there is a transient period of dynamic readjustment which leads to an interval of relative stability. This is followed by a period in which there is a more obvious progressive failure of the circulatory system to terminal levels. In general the initial period is characterized by a return of the several variables towards the calibration level. This is especially evident in the FP and $\frac{1}{2}$ PF values for dog 13 which fell well below the calibration level during the reinfusion and then gradually returned towards the calibration value. It is difficult to be certain just what accounted for the fall in these values. Although the prolongation of the FP and $\frac{1}{2}$ PF intervals is suggestive of a vasodilator response to the reinfusion, it should be noted that the FPP/CPP ratio remains near 1.0. This indicates an absence of any significant reflected component and suggests that femoral pulse contour must be explained in terms of the form of the transmitted wave. Such an explanation is reasonable since the period of reinfusion is characterized by a great augmentation of the central pulse due to the large increase in stroke volume.

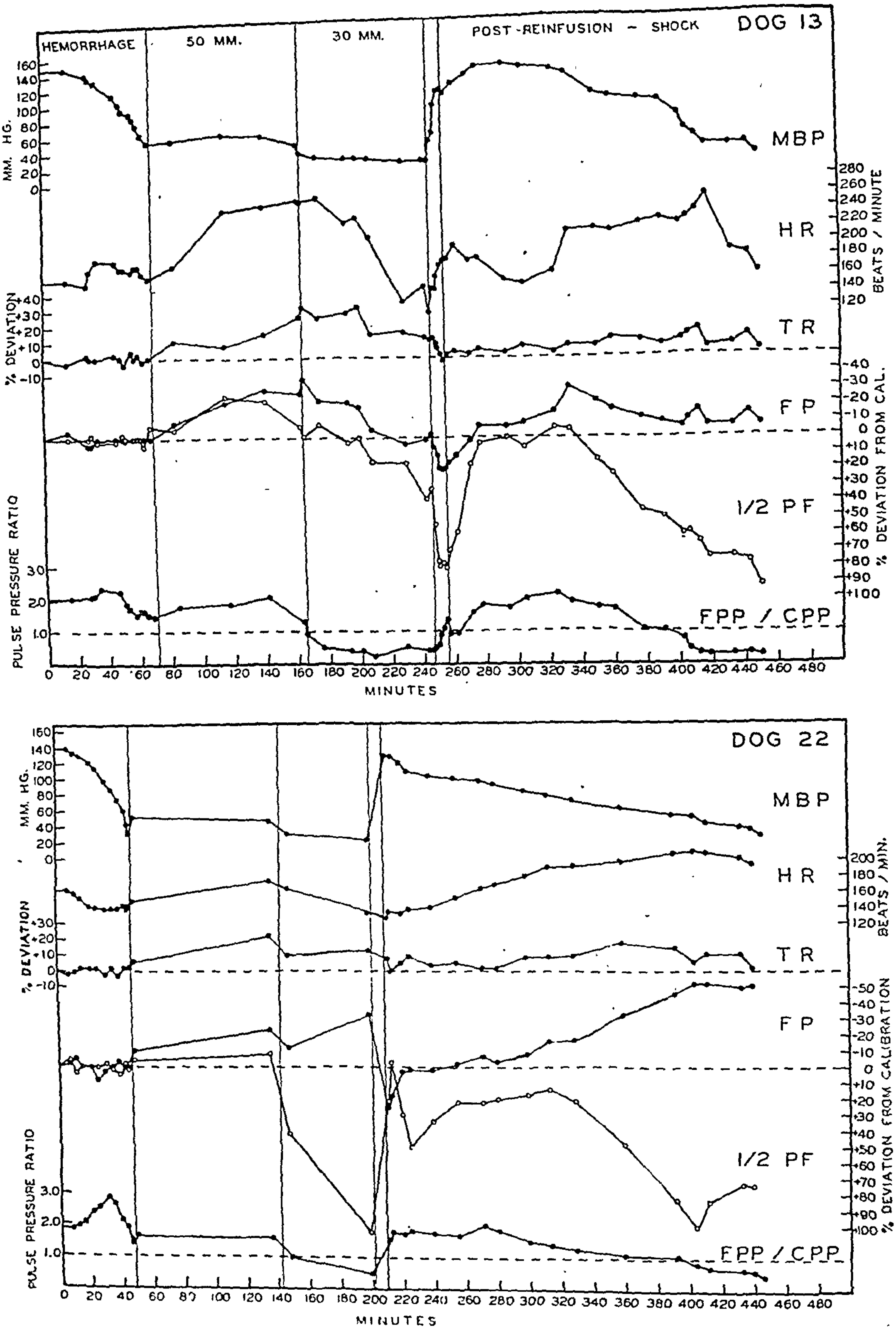

Fig. 4. Analysis of data from dogs 13 and 22. Plotted from top to bottom: Mean blood pressure (*MBP*), heart rate (*HR*), transmission rate of the pulse (*TR*) as per cent deviation from calibration values, duration of the anacrotic limb of the femoral pulse (*FP*) in solid circles and duration of the first half of the catacrotic fall ($\frac{1}{2}$ PF) in open circles expressed as per cent deviation from calibration values, and ratio of femoral pulse pressure to central pulse pressure (*FPP/CPP*).

After this initial period of readjustment the FPP/CPP ratio rises significantly above 1.0 and the interpretation of the peak of the femoral pulse as being determined by the reflected component again becomes valid. During this phase and continuing throughout almost the entire period of circulatory failure it will be noted that heart rates accelerate progressively and at the same time the transmission rate of the pulse remains somewhat above the calibration level. If we employ these two factors as indices of the activity of the cardiovascular compensatory mechanisms responding to a falling blood pressure, it is evident that there is no sign of any generalized failure of these mechanisms until shortly before death. The fact that transmission rates exhibit a somewhat greater degree of compensatory activity than during the calibration period of hemorrhage may be due to the added stimulant of acidosis on the vasomotor centers; or it might possibly represent the action of vasoconstrictor substances which some investigators claim are liberated in shock (18). The contour of the anacrotic limb also fits the picture of an increased volume-elasticity coefficient of the arterial tree due to compensatory constrictor activity, since the FP interval is shortened, rising quite significantly above the calibration level in the case of dog 22. These observations would suggest a shift towards a femoral pulse of type II as observed early in hemorrhage.

The course of the ½ PF interval, however, is in marked contrast to this trend. As indicated above, the significant downward trend of these values during the 30 mm. period is difficult to interpret because of the greatly reduced femoral pulse pressure and consequently a pulse form of type IV. Yet after reinfusion when the reflected component has again become prominent as evidenced by the higher FPP/CPP ratio, there is still a marked abnormality in the catacrotic limb of the pulse. In dog 13 the ½ PF interval returns to the calibration level for a short period after reinfusion and then as mean blood pressure deteriorates these values become greatly prolonged, falling far below the calibration level. In dog 22 the recovery of the ½ PF values after reinfusion never does reach the calibration level and towards the latter part of the experiment there is a great prolongation of the catacrotic fall in pressure.

The consequence of these trends on the pulse contour may be appreciated by inspecting the actual tracings. In figure 5 are shown three pairs of recordings from dog 28 arranged so that pulse contours in shock may be compared with the pulse contours at roughly equivalent pressures during the initial period of hemorrhage. Dorsalis pedis pulses were recorded in this experiment in addition to the aortic and femoral pulses. In records A–B and C–D the changes are not marked, but a more rapid rise of the femoral pulse together with diminished dicrotic waves is evident in B and D. In records E and F the contrast becomes marked, the femoral and dorsalis pedis pulses in shock showing a reasonably sharp rise of the anacrotic limb followed by a gradual fall of the catacrotic limb which is free of dicrotic waves. In figure 6 are presented a series of "before" and "after" recordings from several other animals. Although these pulses show some minor variations, it may be observed that the distinguishing characteristic of the shock pulses is the prolongation of the catacrotic fall with a reduction

in or obliteration of the dicrotic waves. The anacrotic rise, on the other hand is reasonably rapid in all shock pulses except that of dog 5. The femoral pulse in this latter instance will be recognized as an extreme example of the type III pulse; its significance in this experiment will be discussed below.

In table 1 are presented summary data for the 13 animals that responded to the reinfusion to illustrate the uniformity with which these trends were observed

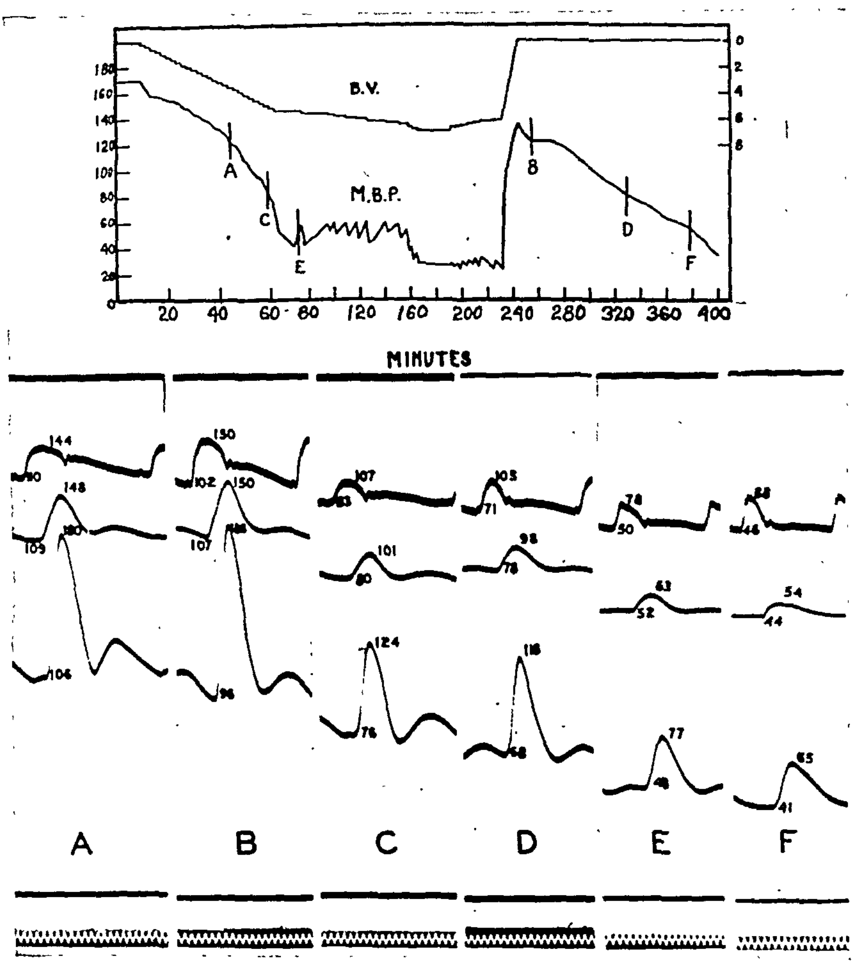

Fig. 5. Recordings from dog 28 arranged so that pulses during shock may be compared with pulses during hemorrhage. Upper graph as in figure 1; tracings below show aortic pulse (upper), femoral pulse (middle), and dorsalis pedis pulse (lower).

in shock. For this table two points were selected: the point at which the pressure became reasonably stabilized after the reinfusion and the point at which the progressive circulatory failure had lowered the mean blood pressure to approximately 80 mm. This latter point was selected arbitrarily as representing a moderately advanced stage of the shock process without encroaching on the terminal periods of extreme hypotension where a variety of pre-mortal changes may enter to complicate the picture.

These animals have been arranged in the order of their survival time, the first nine representing reasonably typical post-reinfusion circulatory failures of varying rapidity. Dog 17 was somewhat atypical in that it showed early signs of rapid failure and then lingered at hypotensive levels for four hours before it exhibited a secondary fall to terminal levels. Dog 12 exhibited a protracted failure that did not conform to the typical picture of hemorrhagic

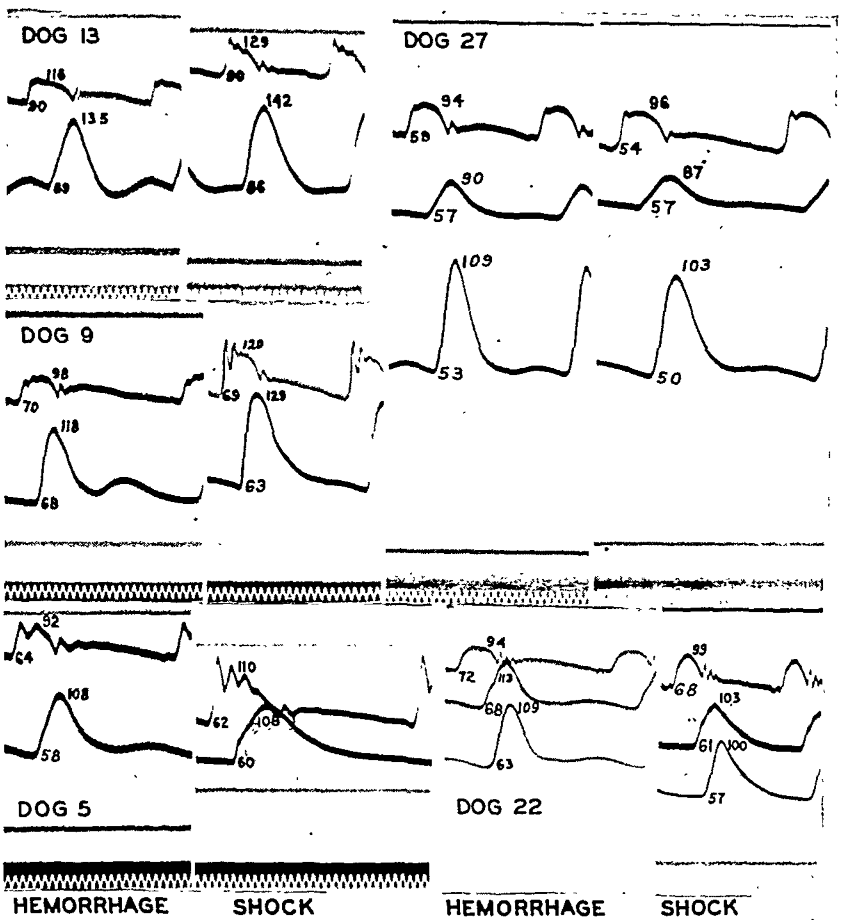

Fig. 6. Recordings obtained during hemorrhage compared with recordings during shock for several animals. Tracings on left show aortic pulse (upper) and femoral pulse (lower); tracings on right show aortic pulse (upper), femoral pulse (middle), and dorsalis pedis pulse (lower).

shock as produced by this procedure, although a diagnosis of shock at the time it was sacrificed was warranted. Dogs 20 and 10, on the other hand, displayed no indications of shock physiologically and autopsy findings were negative even though they were submitted to the same standard bleeding and reinfusion procedure. The very gradual fall in pressure seen in dog 20 during the five hours after reinfusion was attributable to a persistent oozing of the heparinized blood from the surgical incisions which we were not able to control.

Inspection of the data for these animals reveals that with rare exceptions the transmission rate of the pulse is accelerated after the reinfusion and remains elevated throughout the post-reinfusion period. Although some of the individual values are not statistically significant they consistently exhibited general trends of acceleration such as those illustrated in figure 4. Similarly, the time required for the rise of the anacrotic limb (FP) was less than the cali-

TABLE 1

DOG NO.	MIN. AFTER REINFUSION	MEAN BLOOD PRESSURE	PULSE PRESSURE RATIO: FPP/CPP	TRANSMISSION RATE: TR	ANACROTIC LIMB: FP	CATACROTIC LIMB: ½ PF	SURVIVAL AFTER REINFUSION
							(Min.)
6	7	88	1.72	+2	−21	+52	46
	10	76	1.57	0	−15	+51	
5	18	147	2.46	+7	−5	+24	90
	58	85	1.51	+15	−10	+4	
11	5	106	1.72	+2	−21	+40	112
	18	79	1.58	+3	−48	+82	
9	8	87	1.36	−2	+21	+30	136
	82	80	1.23	+15	−16	+14	
27	5	78	0.91	0	+29	+56	160
28	9	126	0.96	+4	+3	−9	190
	88	82	0.59	+4	−11	+19	
13	16	140	1.73	+5	−7	+4	205
	142	78	0.76	+13	−6	+60	
18	8	105	1.42	+6	−21	+37	208
	55	78	1.17	+4	−6	+14	
22	15	110	1.86	+9	+2	+50	241
	103	80	1.41	+9	−16	+22	
17	5	121	1.54	+4	+31	+63	300
	42	78	1.82	+10	+45	+80	
12	13	147	2.15	+6	−13	+26	Sacrificed at 330 min.
	289	81	1.04	+1	−41	+60	Blood pressure: 60 mm.
20	15	140	1.31	+7	+42	+32	Sacrificed at 300 min.
	285	98	1.65	−4	−3	−2	Blood pressure: 95 mm.
10	19	127	1.76	−4	+13	+16	Sacrificed at 270 min.
	260	116	1.91	+5	−4	0	Blood pressure: 118 mm.

Summary data for the 13 animals for whom reasonably adequate post-reinfusion periods were obtained. Transmission rates and the durations of the anacrotic and catacrotic portions of the femoral pulse are expressed as per cent deviation from the calibration values as described in text.

bration value in most instances. Definite exceptions to this often were observed for transient periods immediately after reinfusion, a response which appeared to be especially marked in the case of dogs 10 and 20 which did not develop shock. In dog 17 this post-reinfusion increase in the FP interval persisted down to the 80 mm. level, but shortly beyond this point the interval shortened so that it averaged about 25 per cent less than the calibration value throughout the latter course of the circulatory failure. In contrast, except for a brief

period of shortening observed immediately after reinfusion in dog 28, the ½ PF intervals show a prolongation in all of the animals that developed shock; in most instances this was a very significant prolongation. It is therefore of particular interest to note that dogs 10 and 20 which did not develop shock exhibited ½ PF values that were prolonged for a brief period immediately after reinfusion but then returned to the calibration level. More than four hours after the reinfusion the duration of the ½ PF intervals in these two animals that did not develop shock was essentially the same as that recorded in the calibration data during the initial period of hemorrhage.

These data demonstrate that in most respects the animal in shock exhibits a femoral pulse contour that shows fully as much evidence of compensatory vasomotor response as that seen in simple hemorrhage, but the shock pulses differ significantly from the hemorrhage pulses in the prolonged slow fall of the catacrotic limb. Since in most cases this change occurs with an FPP/CPP ratio that is significantly over 1.0 (table 1) and since it is accompanied by a disproportionate loss of the dicrotic waves (fig. 6), it would appear that this change would probably be related to an alteration in the form of the reflected component of the pulse. To verify this, it is necessary to carry out the algebraic subtraction of the transmitted fundamental wave from the femoral pulse to determine the actual contour of the standing waves created by the summated reflections, a procedure that is simplified by employing the coordirectograph devised by Green and Maurer (7). As indicated earlier, the transmitted component was assumed to be the central pulse with rapid oscillations damped out, a procedure which appears valid as long as one avoids levels of significant hypotension where transmission involves gross damping of the central pulse contour. The actual reflected components determined in this manner for dogs 13 and 22 are shown in figure 7. The solid lines indicate the reflected components of the shock pulses and are to be compared with the dotted curves which are reflected components found in pulses at approximately the same pressure values during the hemorrhage period of these animals. This comparison reveals that the standing wave created by the reflected component in shock rises about as rapidly and at least as soon as that in simple hemorrhagic hypotension, but the initial positive wave has a somewhat prolonged fall followed by a protracted and excessively damped negative oscillation. This change in the contour of the reflected component can obviously explain the distortion that has been observed in the contour of the femoral pulse itself.

Discussion. Attempts to attribute the declining blood pressure in shock to an alteration in the properties of the vascular tree have not been very convincing. Evaluations of the changes in total resistance to blood flow (23, 25) have failed to reveal any uniform evidence of a deficiency in the response of the vasomotor system. These results must be accepted with some reservations since they have referred the resistance in shock to the resistance of the normal animal without adequate consideration of the compensatory change in resistance that should be anticipated with a lowered blood pressure. A suggestion that there may be some deficiency in this compensation (23) has been supported by

some unpublished observations of our own employing the stepwise hemorrhage technique. Some investigators have claimed that vasodilator substances appear in the blood of shock dogs (18), but this finding still awaits adequate confirmation. Other authors have raised the question as to whether there may be some disturbance in the autonomic regulators of the vasomotor system, since intense afferent stimulation increases the mortality of dogs subjected to hemorrhage (14). However, careful investigations of the blood flow to specific organs have failed to uncover any evidence of vasomotor failure in the spleen (13), kidney (16), or hind leg (4). These observations indicate that even though the course of shock may be influenced by the activities of the autonomic vasomotor regu-

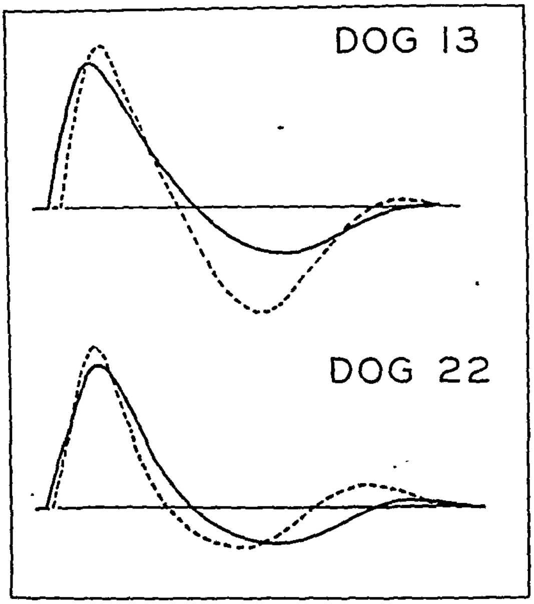

Fig. 7. Standing waves produced by the summated reflections in shock (solid lines) compared with the standing waves produced by the summated reflections at equivalent pressures during simple hemorrhage. Determined by direct co-ordirectographic subtraction of the transmitted pulse from the lateral femoral pressure pulse.

lators, the circulatory collapse cannot be explained as a generalized failure of this system.

This does not exclude the possibility that shock inducing procedures may precipitate a generalized vasomotor failure in specific instances. An example of this we believe to be represented by dog 5. The data in table 1 indicate that 58 minutes after reinfusion dog 5 was maintaining a pressure of 85 mm. Hg and exhibiting a reasonably typical shock pulse with an abbreviated anacrotic limb and a slightly prolonged catacrotic limb. Eight minutes later respiration suddenly failed even though blood pressure was still slightly over the 80 mm. mark. Although artificial respiration was immediately instituted blood pressure fell rapidly and death occurred 24 minutes later. This type of death could be accounted for by a sudden failure of the cerebral mechanisms governing respiratory and cardiovascular function, presumably due to injury incurred by

these centers during the hypotensive period. The bizarre femoral pulse contour for dog 5 shown in figure 6 was obtained shortly after artificial respiration had been well established. This pulse would obviously be classified as type III (fig. 2), the type which was predicted to occur in instances where there was a loss of normal tone of the central arterial vessels. That such a loss did occur in this instance was evidenced by an accompanying reduction in the rate of transmission of the pulse, which dropped from a rate significantly faster than the calibrated value to a rate considerably slower. The association of femoral pulse contours of this type with generalized vasodilatation has previously been demonstrated in the vasodilator response to acetylcholine (8, fig. 3-D) and to nitroglycerine (15, fig. 3-A). Similar changes in pulse contour with a slowed transmission rate of the pulse were observed in two instances of fulminant shock where there was also good grounds for suspecting vasomotor failure.

Typically, however, the femoral pulse in shock does not correspond with this vasodilator type III nor is there a slowing of the rate of pulse transmission.

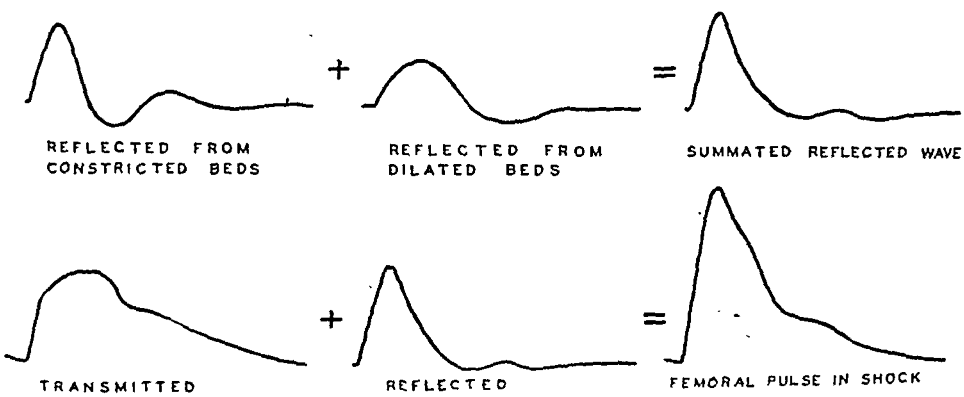

Fig. 8. A possible schematic synthesis of the femoral pulse observed in shock

This latter observation is of particular importance since dilatation of the aorta and central arteries has been suggested as a possible cause for the initiation of a decline in blood pressure in shock (21). A test of this possibility was attempted by Wiggers et al. (24) using the aortagraphic technique, but technical difficulties forced them to abandon the investigation without obtaining conclusive results. This possibility is now excluded by the data for pulse transmission rate plotted in figure 4 and tabulated in table 1. Not only is there an absence of any significant slowing of these rates, but in most cases there is a definite tendency for the rate to accelerate during the period of circulatory failure to values in excess of these observed at equivalent levels of simple hemorrhagic hypotension. It is therefore clear that a process of dilatation of the aorta cannot be called upon to account for either the abnormal pulse contours or the decline in blood pressure in the typical case of shock.

Nevertheless, some explanation must be found for the distortion observed in the contour of the catacrotic limb of the femoral pulse in shock. As shown in figure 7, this distortion is produced by an altered reflected component that

creates a standing wave combining a rapid initial rise followed by a highly damped low frequency oscillation. It must therefore relate to an abnormality somewhere in the arterial tree. Our present knowledge of the detailed dynamic characteristics of the arterial tree are not adequate to draw any very positive conclusions as to the significance of this form of wave. Yet from the standpoint of wave synthesis one can theoretically explain the genesis of such a wave as shown schematically in figure 8. The combination of a rapid oscillation with a slower oscillation may yield a summated standing wave in which a rapid rise is followed by a gradual fall relatively free of oscillations. The further summation of this standing wave with the transmitted wave yields the femoral pulse form indicated. Obviously, such a simplified treatment can hardly be expected to yield an accurate facsimile of the pulse form actually recorded in the dog, but this synthetic pulse does portray the basic characteristics of the shock pulses.

This form of analysis leads to the hypothesis that in shock certain vascular beds are constricted so as to give rise to rapid reflections while other beds are dilated so as to produce slower reflected waves. More specifically, since the reflected component in the femoral pulse arises from beds below the thorax (9) and since this reflection has arrived back at the aorta at a time when the foot of the pulse wave has just started to enter the femoral bed, it follows that a major source of this reflection must reside in arterial beds lying somewhere below the diaphragm and above the legs,—that is, within the abdomen. Among the beds within this region rapid reflections are to be expected from the kidney and spleen which have already been shown to maintain good constrictor tone throughout the course of circulatory failure in hemorrhagic shock (16, 13). This leaves the intestine as the major abdominal structure whose vascular status might be able to account for the low frequency portion of the reflected component in the femoral pulse in shock.

It has long been recognized that one of the most characteristic findings in shock in the dog is a massive congestion of the intestine, particularly of the duodenum where secondary petechial hemorrhages are often found. Zweifach et al. (26) have made microscopic observations of one portion of this bed during the course of hemorrhagic shock. They report the development of a hyporeactive stage characterized by venous congestion, dilatation of the arterioles, and a depression of vasomotion and epinephrine sensitivity of the precapillary elements. As a consequence stagnation and pooling of blood was observed in the capillary bed. Analysis of the dynamics of the intestinal circulation in shock have led to similar conclusions. Wiggers, Opdyke, and Johnson (22) found a consistent elevation of portal pressure in shock, suggesting, a compensatory constriction of the hepatic bed without an equivalent constriction of the mesenteric bed. The implications of this finding were extended by Selkurt and his associates (17) who demonstrated that the resistance to blood flow in the mesenteric circuit in shock did not show a compensatory increase to keep pace with the elevated hepatic resistance. Moreover they demonstrated that in an otherwise normal dog an elevation of portal pressure in itself produces a

dilatation of the mesenteric blood vessels. The inference that under such conditions there would be a pooling of blood in the intestinal blood vessels has been supported by the recovery of an abnormally high residual blood volume from the abdominal region of dogs dying in shock (5). There is good justification, therefore, for attributing the low frequency component in the summated reflected wave of the femoral pulse in shock to a local failure to maintain compensatory constrictor tone in the intestine. The reflections from this dilated bed when summated with reflections from other beds in which there is no failure to maintain constrictor tone can account for the observed distortion in the form of the catacrotic limb of the femoral pulse in shock.

Acknowledgment. This study constitutes one of a large series of investigations into the shock problem under the direction of Dr. Carl J. Wiggers. The authors are greatly indebted to Doctor Wiggers for his many valuable suggestions as to the design of the experiments and the analysis of the data.

SUMMARY

1. By optically recording arterial pressure pulses in dogs during a stepwise hemorrhage, a period of maintained hypotension, and then during the period of circulatory failure following reinfusion of all withdrawn blood, it has been possible to compare the pulse tracings during the shock induced by this procedure with tracings at equivalent pressures during simple hemorrhagic hypotension.

2. The lateral pressure pulse in the femoral artery is assumed to represent the summation of a transmitted pulse with a reflected wave system, the form of the latter being dependent upon the elastic properties of the arterial tree. An initial sharpening of the peak of the femoral pulse and a relative augmentation of femoral pulse pressure observed early in hemorrhage is related to a change in the reflected component due to elasticity changes produced by the compensatory vasoconstriction accompanying the hemorrhage. A secondary reduction in elastic tension is evidenced as further bleeding significantly reduces the volume of blood in the arteries and leads to their relaxation.

3. By "calibrating" each animal for these changes in pulse form with a lowering of blood pressure, it has been possible quantitatively to assess the comparable changes in these details of pulse contour during the course of circulatory shock. On the basis of pulse transmission rates and the values for the duration of the anacrotic rise of the femoral pulse, it is concluded that there is no generalized failure of the compensatory vasoconstrictor mechanism to a lowered blood pressure; rather these values reflect an even greater compensatory response than during hemorrhage. In contrast, the catacrotic limb of the femoral pulse exhibits a marked prolongation in shock with a reduction in the dicrotic waves which does not conform with the type to be expected with a heightened constrictor tone.

4. Since this change in pulse contour appears to be due to a change in the form of the reflected component rather than to a change in the transmitted component, it demands a reflected component which in simplest terms may be

conceived as the summation of a rapid component from constricted beds with a slow component reflected from dilated beds. It is suggested that a local failure of compensatory constrictor tone in the intestinal arterial bed can most logically account for this reflected component of low frequency which leads to the distortion of the normal femoral arterial pulse contour.

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THE EVALUATION OF THE WORK OF THE HEART[1]

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The work of the left ventricle is usually calculated as the sum of two components, one the work done against pressure and the other the work done in imparting velocity to the aortic stream. The work against pressure is PQ, where P is the pressure increase imparted to the blood by the heart, expressed in grams per square centimeter, and Q is the volume of blood ejected. The work done in accelerating the blood is $\frac{MV^2}{2g}$ where M is the mass of the blood, V its velocity in centimeter/second, and g the gravity constant.

The use of this formula has necessarily been subject to simplifying assumptions. It was recognized by Frank (1) that the terms P, Q and V should be integrated over the time of systole. This is possible for P, which can be determined from the central pressure pulse; while Q and V—with some reservation—can be taken from the cardiometer curve. This approach was applied by Straub (2) for PQ, and by Katz (3) for both terms.

It is not expedient to carry out a laborious integration, and in many experiments, where the chest is closed and cardiometer tracings cannot be taken, such a construction is impossible. For this reason the calculation is usually made on the basis of simplifying assumptions. For instance, it is often held that the work done in imparting velocity to the issuing stream is but a negligible part of the total work, and can be neglected. That such an assumption is hazardous might be adduced from the large values for velocity work obtained by both Evans (4) and Katz (3) when the stroke volume became even moderately large. Both workers, however, measured velocities through rigid glass cannulae whose size was not related to that of the aorta during systole. The cross-sectional area of the aorta changes during a pulse beat, a factor which must influence strongly the ejection velocity.

Simplifying assumptions have also been made in calculating the work done against pressure. Some have assumed that it is adequate to calculate this quantity by multiplying the mean arterial pressure by the stroke volume. Others make the quantitation somewhat more accurate by multiplying the mean pressure during systole by the stroke volume.

We have recently published a method (5) by which, in the dog, the course of ventricular ejection can be derived from the pressure pulse contour. The method can be applied in the absence of major surgical procedures with the animal essentially normal. By this method pressure, flow and aortic blood velocity can be determined at 10 msec. intervals. Summation of the calculated work figures

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for these intervals gives results not appreciably different from the results of graphic integration.

It is our purpose to evaluate the errors resulting from the use of the simpler methods of calculating the work of the ventricle by comparing such results with those got by summation.

1. *The method of calculating the work of the left ventricle.* In order to calculate the velocity obtaining from instant to instant in the aorta, the relation between pressure and cross sectional area of the ascending aorta must be known. Such a relation is plotted in figure 1 from measurements previously made (5, 6). A

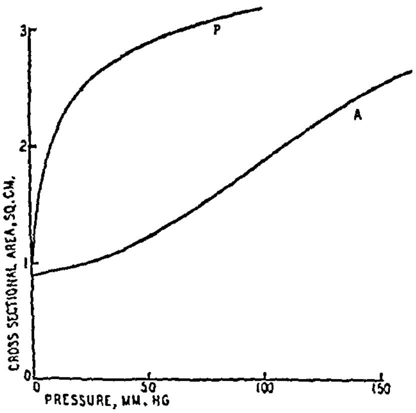

Fig. 1

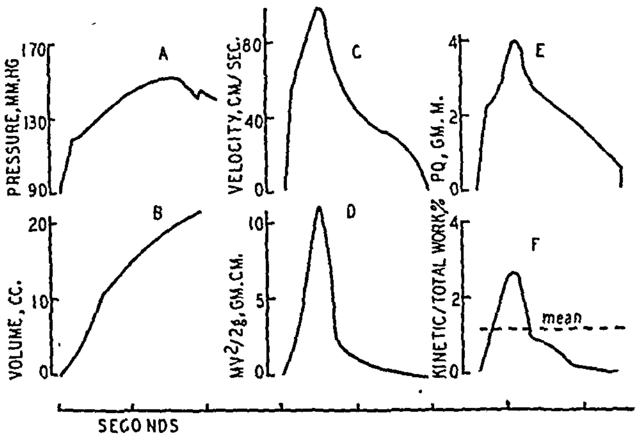

Fig. 2

Fig. 1. Plot of the distensibility of the ascending aorta and of the pulmonary artery.

Fig. 2. The analysis of cardiac work: A, systolic portion of aortic pressure pulse; B, ejection curve derived from A; C, aortic velocity during systole; D, work done in accelerating blood during systole; E, work done against pressure during systole; and F, relation between kinetic and total work.

representative normal pressure pulse was then selected and plotted (fig. 2A). From this was derived an ejection curve (fig. 2B) as described elsewhere (5). The velocity curve was then plotted (fig. 2C) at 10 msec. intervals from values obtained by dividing the flow in cubic centimeter/second, by the cross-sectional area of the ascending aorta at the pressure obtaining. From these velocity figures a curve representing the distribution of the kinetic work during systole was derived (fig. 2D), by the formula $\frac{MV^2}{2g}$.

The next step was to evaluate the work done against pressure by multiplying the volume of blood ejected during each successive 10 msec. interval by the existing pressure. The figures are plotted in figure 2E. The percentage of total work represented by kinetic work is plotted in figure 2F. It varies from 0 to 2.7 per cent, with a mean at 1.2 per cent.

2. *The relation of kinetic to total work.* The kinetic fraction varies roughly with the stroke volume and inversely with the diastolic pressure. This last is because

the greater the pressure the wider the aorta and the less the velocity attained during a given ejection. To illustrate the influence of these two factors, we have selected from a large series of dog pulses, those in which the stroke volumes were relatively large, and in which the velocities might also be expected to be large. The first five of these (table 1) are from dogs under morphine sedation, with slow heart rates and large pulse pressures; pulses 6 and 7 are from the response of an animal to an intravenous injection of isopropyl epinephrine[2], an active vasodilator and cardiac stimulant (7). Pulses 8, 9, 10 and 11 were taken immediately

TABLE 1

The determination of left ventricle work for a series of dog arterial pulses

PULSE	Pd	Ps	Pms	$\frac{\text{Pms-Pd}}{\text{Pp}}$	Pmc	$\frac{\text{Pms}}{\text{Pmc}}$	SV	Work, gm. M.		i/j	Kinetic work/total work	
								Summated	Pms × SV		Maximum	Mean
a	b	c	d	e	f	g	h	i	j	k	l	m
1	112	142	138	0.87	126	1.10	10.8	19.9	20.2	0.99	0.014	0.009
2	100	150	143	0.86	127	1.13	18.6	35.7	36.0	0.99	0.023	0.012
3	90	150	138	0.80	118	1.17	25.2	49.0	47.1	1.04	0.027	0.012
4	70	120	107	0.75	99	1.08	16.4	23.8	23.7	1.00	0.030	0.018
5	63	105	93	0.72	77	1.21	17.9	23.6	22.5	1.05	0.050	0.035
6	84	160	137	0.70	112	1.22	24.8	50.4	45.9	1.10	0.105	0.065
7	52	105	92	0.76	72	1.28	37.0	36.8	32.7	1.12	0.084	0.037
8	70	144	132	0.84	118	1.12	37.0	67.2	66.0	1.02	0.062	0.034
9	100	190	171	0.79	133	1.29	21.2	47.8	48.9	0.98	0.034	0.020
10	35	78	74	0.91	57	1.30	23.0	19.5	19.3	1.01	0.060	0.029
11	34	88	72	0.70	56	1.28	24.6	26.0	23.9	1.09	0.095	0.065
12	28	70	47	0.45	33	1.42	18.9	12.9	12.0	1.07	0.105	0.056
13	20	58	39	0.50	27	1.44	14.2	9.0	7.5	1.20	0.092	0.058
14	16	50	31	0.44	22	1.41	11.9	6.5	5.0	1.30	0.080	0.057

b–Diastolic pressure, mm. Hg.
c–Systolic pressure, mm. Hg.
d–Mean systolic pressure, mm. Hg.
e–Mean systolic pressure—diastolic pressure/pulse pressure.
f–Mean pressure over cycle, mm. Hg.
h–Stroke volume, cc.

after the opening of a shunt connecting the abdominal aorta with the vena cava, when the peripheral resistance was suddenly reduced to a very low value. Pulse 9, with its higher pressure values, represents the first beat following opening of the shunt in an animal whose blood pressure had been increased by an intravenous injection of epinephrine. Pulses 12, 13 and 14 are successive pulses taken immediately after transection of the abdominal aorta.

In some cases, the velocity work reaches a momentary maximum of 10.5 per cent of the total work being done simultaneously (col. 1). Since this rate of kinetic work is not maintained, the total kinetic work represents a smaller fraction of the total over the whole of a cycle. In the 9 pulses selected specifically

[2] Kindly supplied by Frederick Stearns and Company.

to represent very large stroke volumes and low pressure values, the mean kinetic work ranges from 2 to 6.5 per cent of the total. In the five normal pulses, with their more physiological pressure values, this value varies from 0.9 to 3.5 per cent.

Hence it can safely be concluded that the work done in accelerating the blood is negligible as compared to the work done against pressure. From this it follows that the height of the ventricular pressure curve can be but little greater than that of the systolic portion of the aortic pressure curve. Gregg *et al.* (8) were therefore justified in taking Hamilton *et al.* (9) to task for interpreting an apparent large excess in ventricular pressure as measuring velocity potentials in the aorta.

It has been stated recently by Ralston and Taylor (10) that since the flow through the aorta is streamline, calculations of the kinetic work of the heart should take cognizance of a parabolic velocity distribution across the diameter of the aorta. These authors then rederived the formula commonly ascribed (11) to Hagenbach, published in 1860, which states that the energy requirement for the establishment of a parabolic distribution of velocity is twice that required to accelerate flow to a uniform velocity. Hence, Ralston and Taylor conclude that the kinetic work of the heart should be derived from $\frac{MV^2}{g}$ rather than the $\frac{MV^2}{2g}$ of the Berroulli Theorem.

This revision of the classical formulation would be permissible if streamline flow—evidence for which seems to be acceptable—should establish the fact that at the throat of a channel the velocities have already assumed a parabolic distribution. No evidence for an instantaneous establishment of a truly parabolic velocity pattern is adduced, and in fact there seems to be general agreement (11) among students of hydrodynamics that a parabolic velocity distribution is attained only after fluid has passed a long way down the tube (transitional flow). At the issuing throat, the influence of boundary retardation upon the mean velocity is insignificantly small so that the most of the issuing stream occupies a main central core which advances at nearly the average velocity. Corroboration of this view can be had by studying the movement of contrast medium out the aorta of the fetal goat ((12), plate IV). The aorta is outlined to its whole width as soon as it is outlined at all. There is no central core of high velocity. On this basis there seems to be no justification for revising the classical formulation.

3. *Work done by the ventricle against pressures.* The calculation of the work done in ejecting blood against arterial pressure, as determined by summation of values at each 10 msec. interval during systole, was given in figure 2E. Values were calculated similarly for each of the pulses given in table 1 (column *i*). The products of the mean pressure during systole (column *d*) and the stroke volume (column *h*), are also given (column *j*).

The use of average pressures usually gives smaller values than the use of summated figures for two reasons. The first relates to the fact that when ejection is most rapid, pressure is often relatively high. Unfortunately, the simpler calculations from mean systolic pressures and flows bear no constant relation to the more accurate result of summation at small intervals. (Without laboriously constructing an ejection curve it is not possible to evaluate this error.) On the

other hand, in all pulses save the last two, taken from an animal bleeding freely through a cut aorta, the difference is within 12 per cent (column k). It has been our practice, therefore, to compute ventricle work from average pressure values, neglecting the error that may be implicit in this procedure.

The second source of error relates to the fact that the systolic contour is variable and there is no simple way of evaluating mean systolic pressure. In some cases the pressure rises nearly vertically at the beginning of systole and the mean systolic pressure is only a little below peak level. In other cases it rises so gradually that the mean pressure is about half way between systolic and diastolic. For this reason the mean systolic pressure can only be measured from an accurate central pressure contour. The figures in columns d and e give the increment of pressure from diastolic to mean pressure in relation to the total pulse pressure. When the systolic contour is nearly square the figure is high (0.9), when it is triangular it is low (0.5), and in some cases when there is a narrow systolic peak the fraction is actually less than this.

4. *The work of the right heart.* The work of the right heart differs from that of the left heart in that it is against the much lower pressure of the pulmonary artery. It is often assumed, with Evans (4), that the pulmonary pressure is $\frac{1}{6}$ of the systemic pressure and the the pressure work of the two ventricles can be calculated by $\frac{7}{6}$ PQ, where P is mean systemic pressure during systole and Q is volume ejected during systole.

The relation between systemic and pulmonary pressures is not constant (13). Agents which reduce the systemic peripheral resistance lower the systemic arterial pressure, while by increasing the blood flow they may actually raise the pulmonary pressure. Inspiration, moreover, increases pulmonary flow and pressure while it may even decrease the systemic flow. A strict calculation of the total cardiac work would therefore entail a measurement of pulmonary pressure and flow.

It is also said that the velocities attained in the pulmonary artery and aorta are the same. This is based on the fact that the post mortem diameters of the two vessels are similar. The distensibilities of the two vessels are very different, however, and if the pressures in either depart much from resting normal values their diameters will diverge and the velocities in aorta and pulmonary artery will be different (see fig. 1). It should be emphasized, therefore, that the use of $\frac{7}{6}$ to correct left ventricle work to cardiac work is only a rough approximation.

5. *Relation of cardiac work to the work done at the periphery.* The peripheral resistance represents a force against which the energy of the blood at the periphery works. Except for minimal potential pressure energy remaining in the venous blood which returns to the heart, all of the pressure energy of the blood in the peripheral arteries is dissipated in friction. The work done in the periphery by the force of the blood is the product of pressure and flow in appropriate units. The figure is 3 to 5 per cent higher if the calculation is made as the summation of the products of pressure and flow at short (10 msec.) intervals than it is if mean pressures and flows are used in the calculation. The difference in-

creases with increase in pulse pressure. The above calculation is based on the assumption that pressure and peripheral flow are proportional. The discrepancy would be greater if we were to accept the evidence of Green *et al.* (14) that flow is proportional to a power of pressure.

The work done at the periphery is decidedly less than that done by the heart. In calculating the work done by the heart it is necessary to multiply the pressures during systole by the volume ejected against these pressures. In calculating the peripheral work the same total volume is multiplied by the pressures obtaining over the whole cycle. If mean flows and pressures are used, the calculated work done by the heart exceeds that done at the periphery in proportion as mean systolic pressure exceeds mean cycle pressure (column *g*). If the calculation is made by summation at small intervals, the discrepancy is somewhat, though not greatly, increased.

From the viewpoint of efficient function as a high pressure reservoir, the fact that the arterial bed is composed of long tubes, which are filled only after the lapse of a transmission time, represents a serious disadvantage. During early systole, blood accumulates in the upper aorta, increasing the pressure against which the heart works. Later in the cycle this blood distributes itself throughout the aorta by surging up and down in a series of highly damped oscillations which produce the standing waves (15). The puzzling thing is that mean pressure over the whole cycle is the same at both ends of the aorta (15), yet the heart does 10 to 40 per cent more work than is done at the periphery.

What becomes of the energy that is measured by the difference between cardiac and peripheral work? It is not lost by friction due to the net forward movement of the blood in the great arteries toward the periphery. If it were, the peripheral mean pressure would be less than that at the root of the aorta, which is not the case. It is suggested, therefore, that it is due to the same forces which cause the damping of the standing wave. The excess ventricular work results in an unbalance of pressure in the two ends of the aorta which is equalized as soon as the standing waves die out. During this time from 10 to 40 per cent of the energy of ejection has been dissipated. The manner in which this energy has been degraded can only be speculated about. Viscous resistance to stretch on the part of the arterial walls and surrounding tissues, incomplete reflection of the pulse wave at the terminal arteries, and viscosity of the blood, as well as other factors, are to be considered. That there should be such a large energy waste in transferring blood from the heart to the periphery is, to us, a rather startling concept.

SUMMARY. The following were computed from central pressure pulses typical of a wide variety of conditions, and from arterial distensibility measurements:

a. The curve of cardiac ejection.
b. The change in aortic cross-section during systole.
c. The velocity curve during systole (a/b).
d. The curve of kinetic work during systole (using c in $\frac{MV^2}{2g}$).
e. The variations in pressure work during systole (a times pressure in aorta).

CONCLUSIONS

The kinetic work is of the order of 2 per cent of the total work except when the stroke volume is abnormally large and the diastolic pressure is abnormally low.

The pressure work is underestimated by 0 to 12 per cent by multiplying the total ejection by the mean pressure during systole.

The work done by the heart in raising the pressure of the blood is 10 to 40 per cent more than that done at the periphery in forcing blood through the peripheral resistance. Energy in this amount is lost as the aortic standing wave is damped out.

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THE RATE OF REMOVAL OF INTRAVENOUSLY INJECTED BROMSULPHALEIN BY THE LIVER AND EXTRA HEPATIC TISSUES OF THE DOG

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Recently two indirect methods for measuring blood flow through the liver have been proposed (1, 3, 4). The "urea method" of Crandall depends upon the fact that this substance is manufactured solely in the liver and that it is not further metabolized but completely excreted in the urine. Therefore, the amount of urea excreted divided by the arterio-venous difference across the liver × 100 is equal to the blood flow through that organ. The bromsulphalein (BSP) method of Bradley, Inglefinger and associates depends upon the supposition that this substance is excreted solely via the liver and that none of the injected BSP is abstracted from the blood by organs outside of the portal circulation.

Before applying this latter method, to obtain liver blood flow in dogs, we set out to test the assumption of exclusive hepatic disposal of BSP by following the disappearance of this dye in the presence and absence of the liver and portal organs.

METHODS. Three types of experiments were executed.

1. Attempts were made to recover quantitatively BSP in the bile during the course of constant intravenous injections of the dye[2]. Prior to starting the constant infusion, a priming dose of BSP was given. The cystic duct was ligated and the common bile duct cannulated just before the experiment.

2. The disappearance from the blood of a single injection of BSP was followed in intact dogs, eviscerated-hepatectomized dogs, and in eviscerated-hepatectomized-nephrectomized dogs. BSP disappearance was studied in the same animals before and after evisceration.

3. Serial BSP levels were determined in intact dogs and subsequently eviscerated-hepatectomized dogs during a constant intravenous infusion of the dye. In the intact state, a priming dose was given to the animal before starting the constant injection; no priming dose was given to the eviscerated preparation.

BSP determinations were performed as outlined by Bradley (1) on the Evelyn Photocolorimeter. Evisceration-hepatectomy was performed by the one-stage procedure of Markowitz et al. (5).

RESULTS. A. *Recovery of bromsulphalein in the bile in dogs receiving a constant intravenous injection of the dye.* The data in table 1 show that only between 23 and 79 per cent of a constant intravenous infusion of the dye could be

[1] These departments are in part supported by the Michael Reese Research Foundation.

[2] We are indebted to Hynson, Westcott and Dunning for the BSP used in these experiments.

recovered in the bile during the injection period. In any particular time period there was no simple relationship between the amount of BSP injected and the amount excreted in the bile. Increasing the rate of injection by 65 to 100 per cent led only to an increase of from 15 to 30 per cent in biliary excretion. The variations in plasma level either were negligible or so small that they could not account for the BSP which was not excreted.

B. *Removal of bromsulphalein from the blood after a single intravenous injection in intact and eviscerated-hepatectomized animals.* Intact animals removed BSP from blood rapidly during the first five minutes after a single intravenous injection. After this initial period the blood was cleared of the

TABLE 1

Biliary excretion of a constant intravenous injection of BSP in dogs with cannulated bile ducts

DOG	WEIGHT	PRIMING DOSE OF BSP*	TIME INTERVALS	BSP INJECTED	BSP IN BILE	PER CENT BSP EXCRETED IN BILE	SERUM BSP
	kilo	*mgm.*	*minutes*	*mgm.*	*mgm.*		*mgm. %*
A	8.0	25	0–30	23.5	15.4	65	0.85
			30–60	23.4	14.6	61	0.95
			60–90	23.4	13.1	56	1.05
			90–110	15.6	12.3	79	1.05
B	14.0	37	0–30	34.2	17.4	51	1.3
			30–60	34.2	19.5	57	1.75
			60–90	52.8	23.8	45	2.45
C	20.0	50	0–30	32.1	13.8	43	0.7
			30–60	32.1	11.7	36	0.8
			60–90	64.2	14.7	23	0.84
Average						51.6 ± 16.6	

* The constant intravenous injection was started immediately after the priming dose and the collection of bile began 30 minutes thereafter.

dye at a slower but fairly constant rate. Following evisceration and hepatectomy, whether extremely high or low blood levels were obtained initially, 50 per cent of the dye disappeared from the blood within 50 minutes after mixing had occurred (fig. 1). After the first 10 minutes the eviscerate preparation removed the dye at a more rapid rate than did the intact animal. The dye was not lost in the urine since it was cleared from plasma in eviscerated-hepatectomized-nephrectomized animals at a rate similar to the eviscerated-hepatectomized preparations.

C. *Effect of constant intravenous injections of BSP upon serum levels of dogs before and after evisceration and hepatectomy.* Following evisceration and hepatectomy, 0.7 mgm./minute of the dye was injected and after an initial increase, the serum BSP level became stabilized despite the continuous inflow of the dye (table 2). During this stabilization period, therefore, the peripheral tissues took up or destroyed an amount of dye equal to the amount injected.

treatment 50 to 70 or even a higher percentage was furnished by fat. In some experiments the prethyroid proportion was completely reversed and calories derived from starch reached 80 per cent in the course of thyroxine administration, while before and after treatment 80 per cent was furnished by lard.

Discussion. The thyrotoxic state produced by prolonged administration of thyroxine increased food intake considerably. The fact that this increase might follow the rise in the rate of metabolism after a lag of some days and persisted 5 to 7 days after the sharp decline of O_2-consumption suggests that increased food intake was not directly related to the injection of thyroxine or the resultant rise in the rate of metabolism. Presumably there must be some mechanism

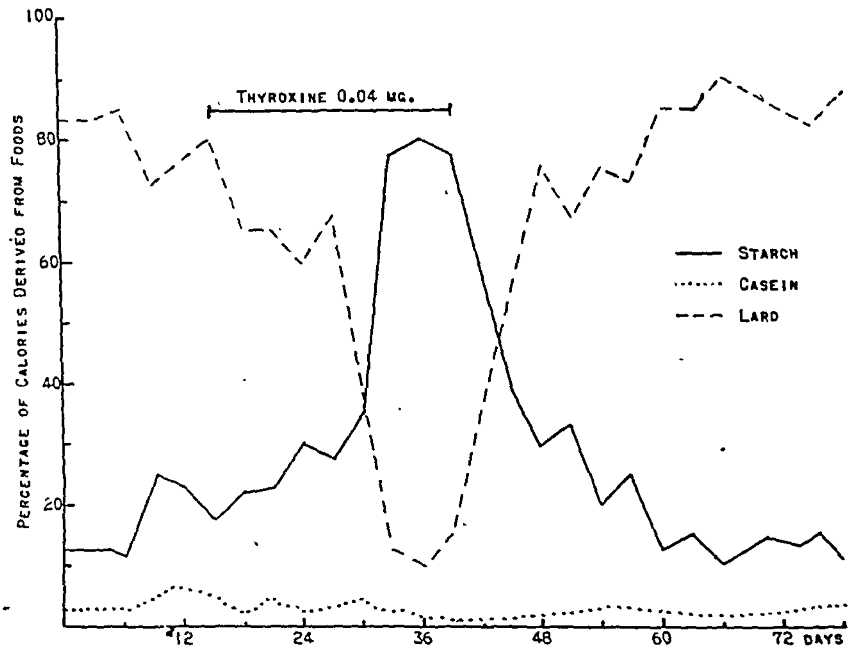

Fig. 4. The effect of thyroxine administration on the proportion of calories derived from different foods.

regulating food intake which is set in motion by the drug directly or as the indirect result of the increase in metabolism.

Experimental evidence presented shows clearly that a very great change in selection was associated with the increase in food intake. During the first days of increased food consumption the qualitative change was less prominent, but later the preference for starchy food became so marked that at the height of the thyroxine effect the additional calories ingested were furnished in many cases almost entirely by carbohydrates. In some cases the consumption of fat fell even below the initial level.

The selective rise in carbohydrate intake is the more remarkable as before and after thyroxine treatment a marked preference for the fatty food was evident. It would seem natural that the necessary additional calories should be

furnished by the food of highest caloric value—at least in an animal having in normal conditions a marked preference for fat.

The fact that prolonged administration of thyroxine is associated with essentially similar—though much more pronounced—changes in food selection as observed in animals exposed to low temperatures (11°C.), may be considered as valuable circumstantial evidence in support of the suggestion advanced in an earlier communication (Donhoffer and Vonotzky, in press) that the selective rise in carbohydrate consumption at low temperature may be due to changes in thyroid function.

Considering the remarkable rise in consumption of carbohydrate-rich food under the conditions described, it seems probable that the composition of food and thyroxine tolerance may be connected. This supposition was suggested by the results of Abderhalden and Wertheimer (1926) and Schlossman (1929) but also by analogy by the observations of Richter and co-workers (1936, 1938), according to which sodium chloride intake of adrenalectomized and calcium intake of parathyroidectomized rats increases considerably. The thesis that an increased ingestion of carbohydrate protects the animal against the damaging effect of long continued thyroxine administration is not supported by the few experiments we have performed to test this hypothesis. Briefly the experiments were as follows. Four groups of 10 mice were fed exclusively diets 1, 2, and 3, or the standard diet, and given thyroxine until death occurred. All showed the same rise in basal metabolism and no significant difference in death rate was evident. Therefore no therapeutic inferences can be drawn from these experiments on the value of dietetic measures in hyperthyroidism.

SUMMARY

Prolonged administration of thyroxine considerably increased the food intake of white mice. The increase in food intake followed the rise of O_2-consumption after a lag of some days and persisted for a similar period after the fall in the rate of metabolism.

The additional calories ingested during the effect of thyroxine were furnished, free selection provided, mainly, in many cases entirely, by consumption of starchy food. At the height of the thyroxine effect this often provided 80 per cent of the calories, while in untreated animals 70 to 80 per cent were furnished by the selection of a fat rich food.

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THE CLEARANCE OF INULIN AND SODIUM P-AMINOHIPPURATE IN THE RAT[1]

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The natural advantages of the rat as an experimental animal have made it worth while to establish reliable methods for the estimation of renal function in this species. In 1942, two of the present authors presented a method using inulin and diodrast which gave reproducible results and was of value in comparative experiments, although admittedly cumbersome (1). Dicker and Heller, in 1945, criticised the absolute value of the method since ether had been used and renal function probably considerably depressed (2). They presented a modified procedure which was, however, still too cumbersome to be of value for large groups of animals. In both these methods, the emptying of the bladder at the start of the procedure and again ten to thirty minutes later entailed a double hazard, since incomplete drainage at the start or at the end of the clearance period would give abnormally high or low clearance values respectively. Further, no attempt had been made to determine whether plasma concentrations of the test substances measured at the end of the clearance period were valid for the whole of that period.

More recently, Meyer Friedman has estimated the clearance of sodium p-aminohippurate (PAH) in the rat (3), but his methods are open to certain criticisms. It seemed to us that a useful clearance procedure should be established on a firm basis with some absolute validity and adapted for large series of animals. P-aminohippurate, whose advantages are well known (4), was used throughout instead of diodrast.

EXPERIMENTAL. *The relation of dose to plasma level.* Preliminary to arranging a satisfactory clearance procedure, the response of the plasma level to administration of the test substance (inulin or PAH) was investigated. This was considered necessary if the test substance were to be given as a single dose without sustaining infusions and if a single blood sample were to serve as a guide to the plasma level during the test period.

a. *The PAH plasma level after injection.* It was hoped that a quantitative picture of the plasma level could be obtained by following a single subcutaneous administration of PAH. Since clearance is expressed in terms of plasma, while such curves could only be studied using small samples of whole blood from the tail, preliminary experiments were performed to determine whether the whole blood analysis could yield the plasma concentration by calculation. The method of Bratton and Marshall for the analysis of PAH was used (5). Male albino rats were injected subcutaneously with small quantities of PAH and samples of

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blood taken by heart puncture. The hematocrit was determined and a sample of blood analysed directly. The remaining blood was then centrifuged and the supernatant plasma analysed. The precipitated red cells were washed with isotonic saline until the washings were free of PAH. The cells were then laked and analysed for their PAH content. Table 1 shows that the plasma level of PAH can be calculated accurately from the whole blood analysis if the hematocrit value is known.

PAH as the aqueous solution of the sodium salt was then injected into normal rats. The desired doses, 30 to 100 mgm., were prepared by diluting the clinical 20 per cent solution in 2 per cent sodium sulfate so that the required amount was contained in 4 cc. This quantity was injected subcutaneously and successive small samples of tail blood were analysed, 0.1 cc. of blood being used for the determination. Hematocrit values were determined at varying times in each experiment. As a final precaution, the PAH content of the final tail blood sample was compared with that of heart blood obtained at the same time. Curves of this type and in this range were constructed for 14 adult male rats.

TABLE 1

BLOOD PAH A	HEMATOCRIT B	PLASMA PAH		CELL PAH	
		Analysed C	Calculated $A \times [100+(100\text{-}B)]$	Analysed	Calculated $A\text{-}\left[\frac{C\text{x } 100\text{-}B}{100}\right]$
mgm. %	*%*	*mgm. %*	*mgm. %*	*mgm. %*	*mgm. %*
2.0	46	3.2	3.1	0.2	0.3
1.5	45	2.3	2.3	0.2	0.3
2.0	46	3.2	3.1	0.2	0.3
2.4	48	3.7	3.6	0.2	0.3

As shown in figure 1, the 40 mgm. dose yields a steady plasma level. In this case, the value at 52 minutes was 5.2 mgm. per cent, while the calculated average for that period was 5.3 mgm. per cent. Doses above 40 mgm. cause the plasma level to rise to a maximum within approximately 30 minutes and then to fall. With smaller doses the peak level is reached more rapidly. Apparently, with doses above 40 mgm. the hippurate enters the blood faster than it can be cleared by the kidneys. Thus, where the dose of PAH administered is of a size to produce a pronounced maximum in the curve, the end point plasma value may not represent the mean. Although the plasma concentration falls less rapidly after 60 minutes, clearance determinations based on end point values obtained even beyond this time still deal with a rapidly falling curve. On the other hand, repeated curves obtained from animals given a 40 to 50 mgm. dose showed that a plasma sample taken 50 minutes after injection was representative of conditions existing over the entire period.

These data indicate that the renal saturation level for PAH lies in the neighbourhood of 6 mgm. per cent. No variation in hematocrit was noted despite the repeated small bleedings and there was close agreement between the last tail

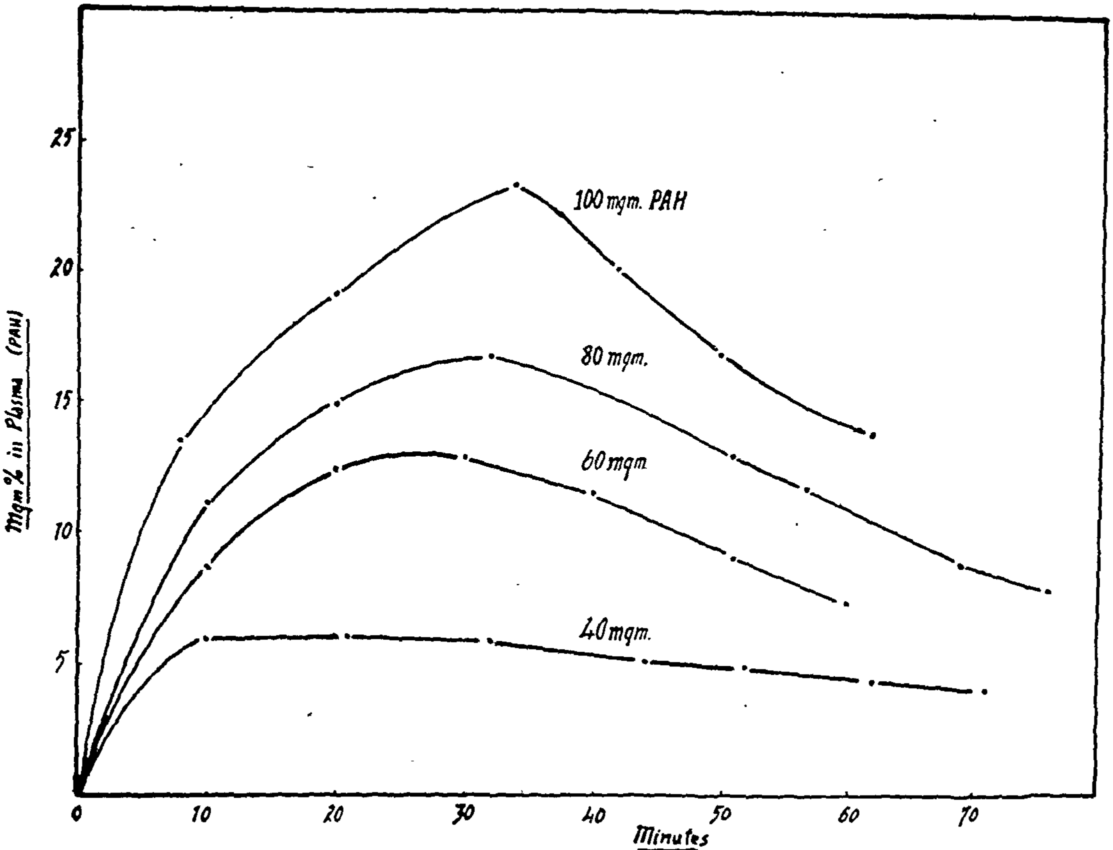

Fig. 1. The relation of the plasma concentration of PAH to the administered dose

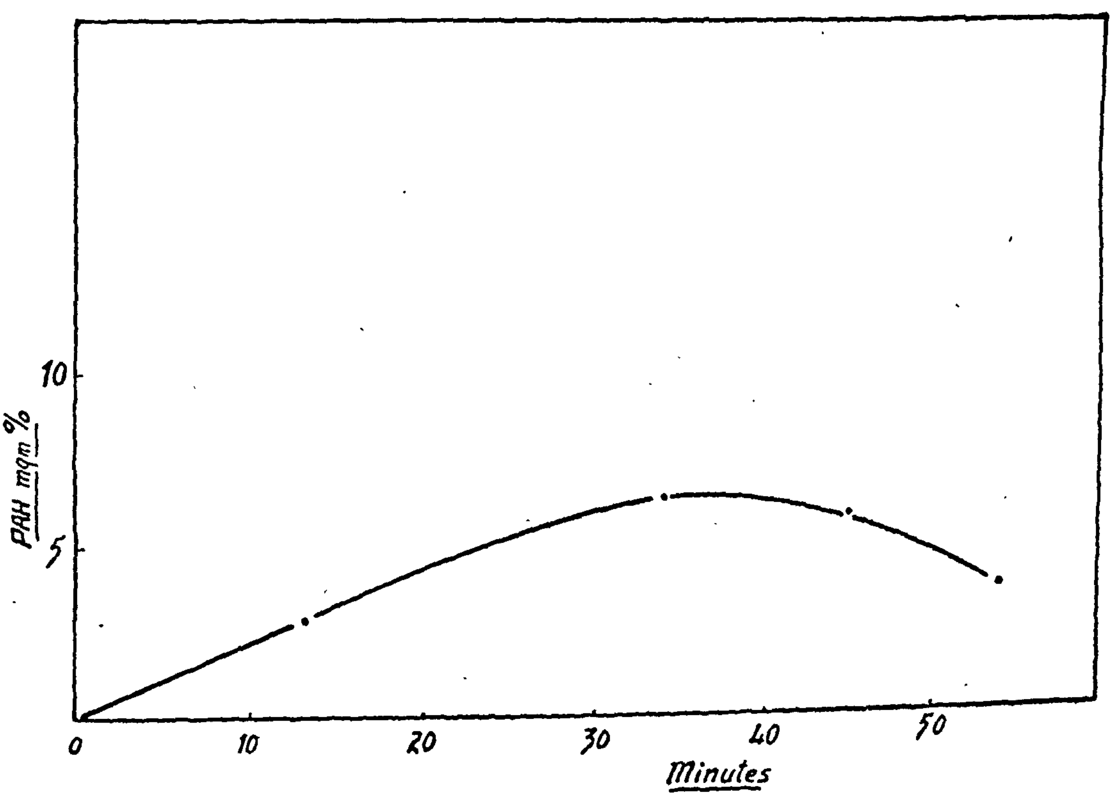

Fig. 2. A type of plasma concentration response to administered PAH

blood analysis and the analysis of the simultaneous heart blood sample. The curve shown in figure 2 indicates that even with low doses, end point plasma values may be misleading. In this animal, the plasma level rose slowly to 6.3 mgm. per cent, then fell rapidly to 3.6 mgm. per cent at 54 minutes, a value not truly representative of the mean. Numerous experiments have shown that when the 50 minute plasma value lies below 4.5 mgm. per cent, the curve may be of this type.

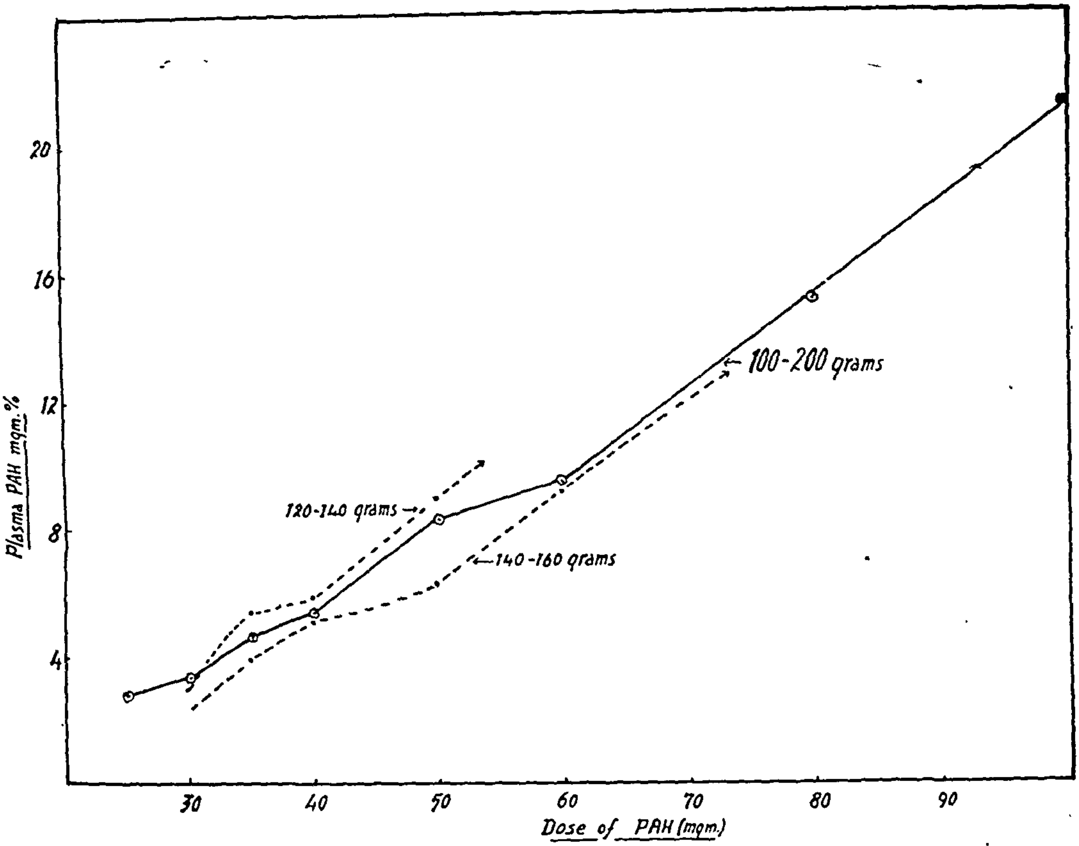

Fig. 3. The relation of plasma concentration 50 minutes after subcutaneous administration of various doses of PAH.

Two conclusions with regard to clearance procedures for PAH using the single dose technique follow from this analysis. 1. If the clearance period begins immediately after injection and lasts 50 minutes, a steady plasma level can be maintained by a suitable dose. 2. If the clearance period starts 30 or 60 minutes after injection, requiring a larger initial dose of PAH, a variably falling plasma level during the clearance period results.

To ascertain the dose of PAH necessary to yield correct plasma levels 50 minutes after injection in different size animals, a large series of determinations was carried out. The data are summarised in figure 3. The plasma concentration 50 minutes after injection is almost linearly proportional to the dose adminis-

tered. The dose is not sharply critical, a difference of 10 mgm. producing a change in plasma level of approximately 2 mgm. per cent. In this composite curve a slight break occurs in the 40–50 mgm. dose range, corresponding to 5–7 mgm. per cent in the plasma. This break, not very apparent in the over all grouping of animals weighing 100 to 200 grams, was very obvious with a more restricted weight grouping. Thus, for animals weighing 120 to 140 grams, and 140 to 160, the curve breaks sharply between 5 and 7 mgm. per cent, a further indication that this is the level of renal saturation.

b. *The inulin plasma level after injection.* As in the case of PAH it was of importance to investigate the variation of the plasma concentration with time after the administration of inulin. This problem was not studied previously because of the lack of suitable chemical procedures. With the publication by Harrison (6) of a method which could measure as little as 2γ of inulin it became feasible to investigate this problem provided that 1, the plasma value could be

TABLE 2

BLOOD INULIN	PLASMA INULIN	
	Calculated	Analysed
mgm. %	*mgm. %*	*mgm. %*
6.5	13.0	14.0
10.0	20.0	20.8
8.0	16.0	15.6
13.5	27.0	26.5

obtained from the whole blood value by calculation, and that 2, in view of the small size of the sample, the necessity for yeast treatment to remove fermentable sugars could be avoided.

It was expected that no inulin would enter the red cell. Thus, assuming a hematocrit of 50 per cent (normal for the rat) the plasma concentration should be twice that of the blood. A series of animals was studied to satisfy this assumption. Inulin was injected, a blood sample taken, and the inulin content of both blood and plasma determined. Table 2 presents typical data, and shows that the whole blood concentration obtained by analysis is directly convertible to the plasma value providing the hematocrit remains unchanged.

In the analysis of whole blood it was found that the inulin content could be estimated without yeast treatment by subtracting a blank blood value obtained from the same animal before the administration of inulin. Indeed, a calibration curve for inulin in blood differed from the aqueous curve only by the blank value of the particular blood used to establish the curve (fig. 4). Investigation of a series of normal animals showed that this blank value was fairly uniform.

A 2 per cent solution of inulin in saline was then prepared, adsorbed on charcoal while hot, and filtered hot through a Seitz EK disc. Three cubic centimeters (60 mgm.) of this inulin solution were injected intraperitoneally to insure uniform and rapid absorption into the blood stream. In our experience inulin

solutions are poorly absorbed from the subcutaneous tissues. The typical plasma concentration curves for a series of animals are shown in figure 5.

From a study of such curves it appeared that the intraperitoneal injection of this amount of inulin yielded a plasma concentration which increased smoothly for at least 50 minutes. The mean value of the inulin concentration in plasma for that period corresponded closely to two-thirds of the value obtained by analysis of a sample taken at 50 minutes.

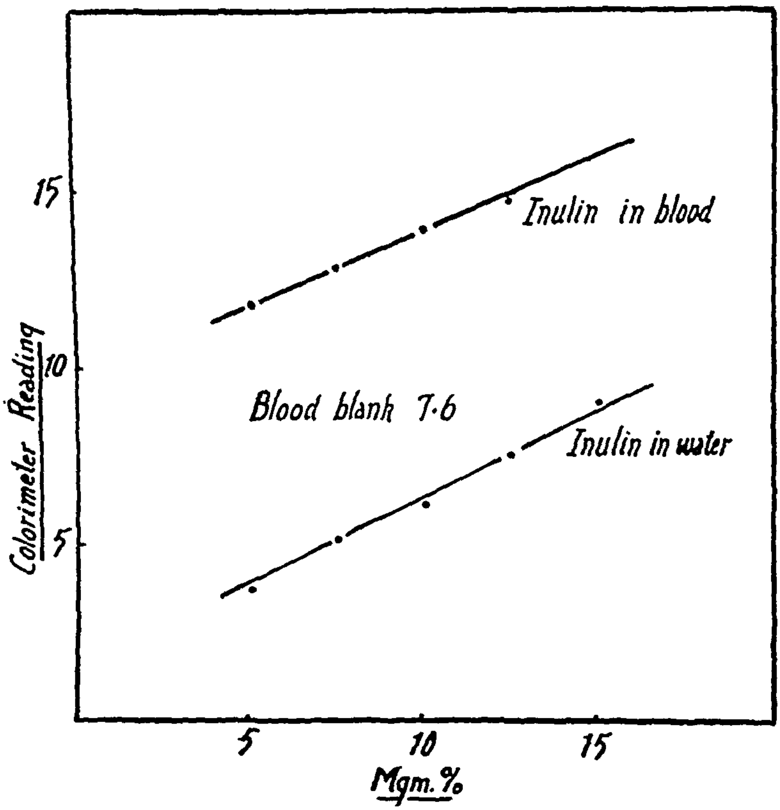

Fig. 4. The colorimetric reading of inulin in blood is the sum of its inulin content plus the blank value for whole blood.

The clearance of inulin and PAH. It became increasingly clear that for the determination of both inulin and PAH clearances, an experimental period extending for 50 minutes from the time of injection was an eminently suitable arrangement. Not only are the plasma concentrations reasonably steady, but in addition, the necessity of draining the bladder at the start of the period is eliminated. This diminishes the hazard of incomplete urine collection. Based on this, a series of clearance determinations were carried out in normal male rats. Although the clearance of inulin and PAH were determined simultaneously, for convenience in discussion they will be separately presented.

a. *The clearance of inulin.* The clearance of inulin, C_{IN}, was investigated in 60 adult rats. The average values obtained were plotted against the plasma

concentration, which ranged from 30 to 60 mgm. per cent. The inulin clearance was independent of plasma level in the range investigated; the average value for C_{IN} when the plasma concentration was 31–40 mgm. per cent was 0.72 cc./100 grams/min., when 41–50 mgm. per cent, 0.75 cc., and when 51–60 mgm. per cent, 0.70 cc. In these experiments urine flow was not excessive, averaging 0.5 to 0.7 cc./hour, and inulin clearance was independent of the rate of urine flow.

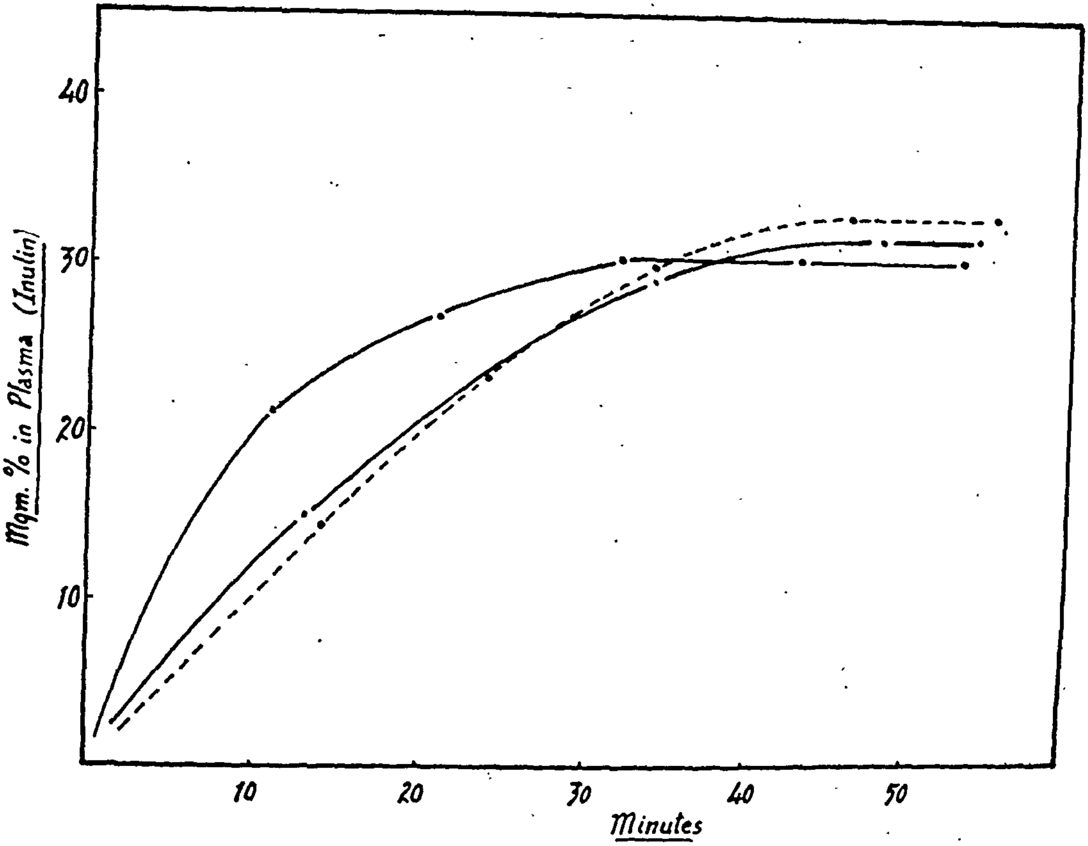

Fig. 5. The plasma concentration of inulin after intraperitoneal administration of an aqueous solution in three animals.

b. *The clearance of PAH.* According to the classical work of Smith and his co-workers (7, 8) the clearance of a substance which is wholly removed from the plasma during one passage through the kidney measures the renal plasma flow. The clearance of this substance will be independent of its concentration in the plasma providing this does not exceed the capacity of the tubules. When the plasma concentration exceeds the saturation level of the tubules, further excretion becomes referrable only to filtration, and the clearance is depressed.

In figure 6 the clearance of PAH, C_{PAH}, is plotted against the 50 minute plasma concentration. The data were obtained from the same 60 animals discussed above. It is evident that the clearance is independent of plasma level where this is not less than 4.5 mgm. per cent nor more than 7 mgm. per cent.

Above 7 mgm. per cent the clearance is progressively depressed while, on the other hand, abnormally high clearance values are obtained when the concentration falls below 4.5 mgm. per cent. This latter finding is interpreted as a confirmation of the caution stated previously, that a 50 minute plasma level below tubular saturation probably reflects a falling curve during the clearance period. Such end point values are lower than the true mean and hence yield falsely high clearance values.

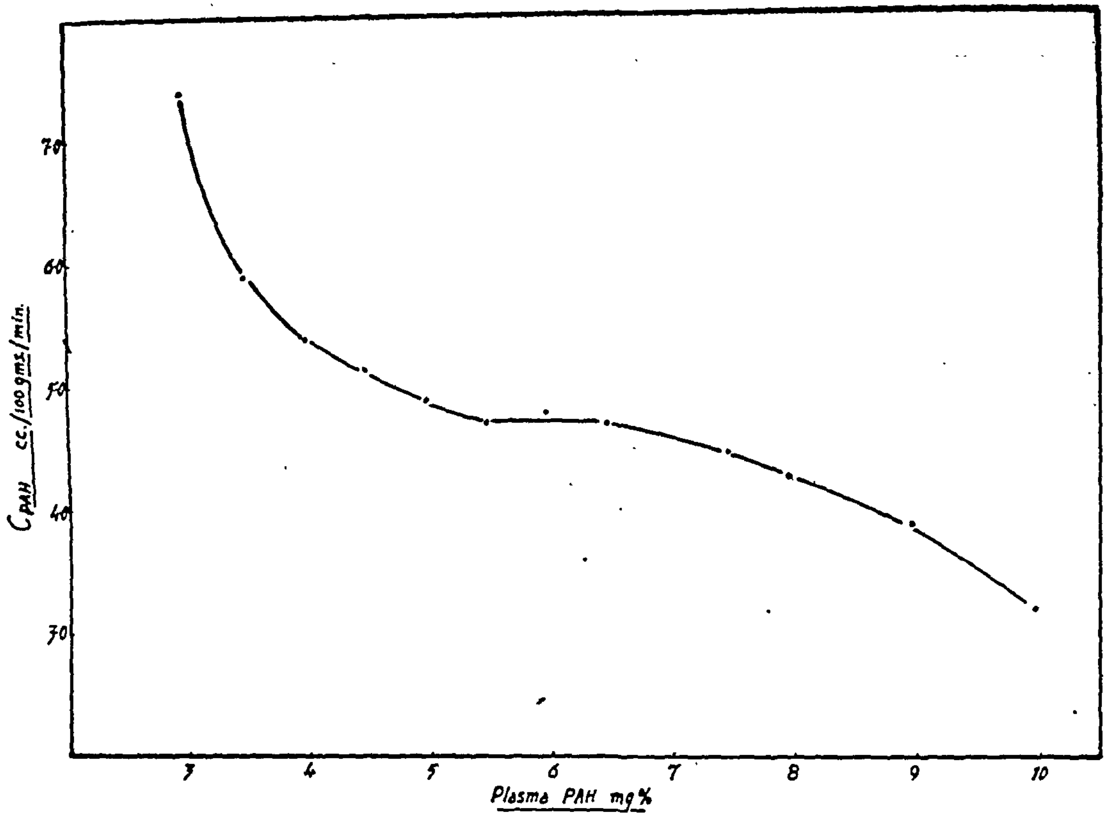

Fig. 6. The clearance of PAH at various plasma concentrations. Each point is the average of determinations in several animals weighing 120 to 160 grams.

c. *The measurement of tubular mass.* The tubular excretion, T, of a test substance is the minute urinary output of that substance less the amount excreted by filtration. In calculating T, since the water content of plasma, W, is relatively constant at about 0.9, it is taken as unity. Similarly, for those plasma concentrations under discussion a negligible error is introduced by considering the freely filterable fraction of PAH, F, to be constant at 0.8 (4).

Once the tubules are saturated, T becomes a constant, Tm, which reflects the amount of functional excretory tubular tissue. As shown in figure 7 the saturation level, where T becomes a constant, is again 5 to 7 mgm. per cent. Beyond 7 mgm. per cent a secondary rise in T is observed. This is expected, since no correction was made for the change in F as the plasma concentration

increased. Moreover, above tubular saturation the curve of plasma concentration for the period is humped and the 50 minute plasma level used in the calculation of T is lower than the actual mean plasma concentration for the period. It is important, therefore, in the study of clearance values in normal animals that the terminal plasma level of PAH lie within a restricted range, if values derived are to have absolute as well as relative meaning.

Procedure for the simultaneous determination of inulin and PAH clearances in large series. In designing a clearance procedure applicable to the study of large

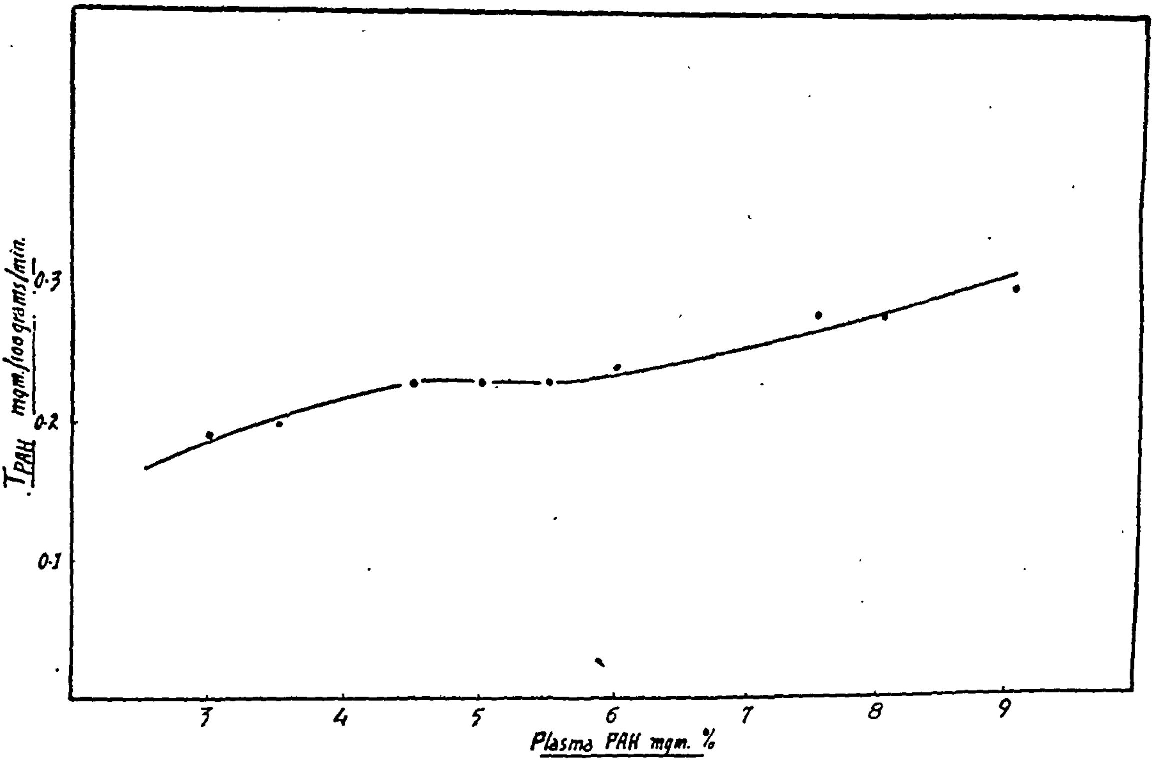

Fig. 7. Tubular excretion of PAH at various plasma concentrations. Each point is the average of determinations in several animals weighing 120 to 160 grams.

series of animals the method should be as simple as possible. To effect this the inulin and PAH analyses were modified so that they could be carried out simultaneously as far as possible. The animal and analytical procedures were as follows.

Animal methods. Four cubic centimeters of PAH solution (12.5 mgm./cc. in 2 per cent sodium sulfate) are injected subcutaneously in the lumbar region. The 50 mgm. dose will yield the correct plasma level in almost all animals of 160 to 210 grams. If the weight is 120 to 160, a 45 mgm. dose suffices, while for animals of 220 to 260 grams the dose may be raised to 55 mgm.

Immediately following this, 3 cc. of warm inulin solution (2 per cent inulin in saline) is injected intraperitoneally. Although the inulin solution in the earlier experiments was treated to remove pyrogen, we have latterly found this unnecessary. The collection period

is timed from the completion of the second injection. The rat is placed directly into a metabolism funnel. Fifty minutes after injection the rat is picked up over the funnel and the bladder drained by suprapubic pressure. Micturition is usually quite free and spontaneous, however. Immediately following urine collection 0.75 cc. of blood is obtained by heart puncture, using a 24 or 25 gauge needle. With practice, the blood is taken during the 51st minute. The animal is then returned to its cage unharmed and the blood centrifuged for 15 minutes at high speed.

The funnel is rinsed thoroughly and urine and funnel washings made up to a total volume of 100 cc., 0.2 cc. of which is used for the analytical procedure. If the diluted urine is heavily contaminated with feces it may be cleared by filtration; under ordinary circumstances, however, it remains reasonably clear. Attempts to filter the urine directly by devices within the funnel are not advisable since there may be the loss of a drop of urine containing a high concentration of the test substances. Urine made up as above yields a negligible blank value for both PAH and inulin despite feces contamination.

Chemical methods. 1. Add 0.2 cc. plasma to 6 cc. distilled water, rinsing the pipette thoroughly.

2. Add 2 cc. of 13 per cent trichloracetic acid. Shake and centrifuge at moderate speed for 5 minutes.

PAH	Inulin
3a. To 3 cc. of supernatant add 0.3 cc. of 0.1 per cent sodium nitrite. Shake and allow to stand for 3 minutes.	3b. Place 2 cc. of supernatant in a boiling tube.
4a. Add 0.3 cc. of 0.5 per cent ammonium sulphamate. Shake and allow to stand for 3 minutes.	4b. Add 4 cc. of Harrison's reagent
5a. Add 1.5 cc. of 0.1 per cent N-(naphthyl) ethylene diamine dihydrochloride. The color develops rapidly. Allow to stand 10 minutes, then transfer to a micro colorimeter tube for reading in a photoelectric colorimeter using a 525 μ filter. The colormeter is set to zero with a colorless reagent blank.	5b. Place the tube in boiling water for 30 minutes, cool at room temperature for 30 minutes, transfer to a micro colorimeter tube and read in photoelectric colorimeter with a 625 μ filter. The colorimeter is set to zero with a reagent blank.

Experiments with rat plasma show a negligible blank for PAH and a small relatively constant blank for inulin where the solutions are free of turbidity. At inulin plasma levels above 30 mgm. per cent the blank value may be ignored.

Urine diluted as stated in the section dealing with animal methods is handled in the same way as plasma, 0.2 cc. being further diluted in 6 cc. of water and exposed to the precipitating agent.

Using this technique, the animals are conveniently handled at the rate of 16 a day by one operator and one analyst.

In calculating the normal clearance values obtained where the terminal PAH level is between 5 and 7 mgm. per cent it was observed that renal function did not increase in direct proportion to body weight, so that expressing results in terms of 100 grams yielded lower values for larger animals, as seen in table 3. This difference disappeared if the results were corrected according to kidney weight using the formula of Braun Menendez (9). The formula, y (kidney weight) = 2.06 x (body weight) + 277, is in reality only a special case of the general power formula $y = ax^b$, where a and b are constants; kidney weight is

actually a function of surface area. Meeh's application of the power formula to surface area in the rat, $y(\text{cm}^2) = 11.23 \text{ weight}^{2/3}$, was applied to the data and table 3 shows the identity of values obtained for the two series of animals when the data were recalculated for 100 cm².

TABLE 3

GROUP	NUMBER OF ANIMALS	WEIGHT	C_{IN} cc./100 g.	C_{PAH} cc./100 g.	Tm_{PAH} mgm./100 g.	C_{IN} cc./100 cm².	C_{PAH} cc./100 cm²	Tm_{PAH} mgm./100 cm²
		gms.						
1	8	142	0.84 ± 0.06	5.02 ± 0.36	0.23 ± 0.01	0.39 ± 0.02	2.32 ± 0.17	0.10 ± 0.01
2	8	177	0.69 ± 0.10	4.40 ± 0.34	0.19 ± 0.01	0.36 ± 0.04	2.31 ± 0.18	0.10 ± 0.01

TABLE 4

NO.	WT.	C_{IN} cc./100 cm²	C_{PAH} cc./100 cm²	Tm_{PAH} mgm./100 cm²	$\frac{C_{IN}}{C_{PAH}} \times 100$	$\frac{C_{PAH}}{Tm_{PAH}}$
					%	
1	155	0.36	2.16	0.11	16.7	19.1
2	154	0.38	2.20	0.10	17.5	21.2
3	146	0.39	2.40	0.10	16.6	22.4
4	153	0.36	2.59	0.10	13.8	25.6
5	147	0.40	2.45	0.11	16.5	22.0
6	148	0.42	2.33	0.12	18.0	19.2
7	140	0.42	2.07	0.10	20.4	19.7
8	140	0.41	2.41	0.10	17.1	24.1
9	175	0.29	2.13	0.09	13.6	22.5
10	168	0.39	2.32	0.10	17.0	22.3
11	186	0.36	2.23	0.10	16.4	22.0
12	188	0.35	2.18	0.08	16.0	23.0
13	175	0.27	2.14	0.10	13.1	21.7
14	168	0.36	2.32	0.09	17.0	25.2
Average		0.36	2.31	0.10	15.7	22.1
S.D.		±0.04	±0.18	±0.01	±2.3	±2.0

In table 4 normal clearance values obtained from 14 animals selected at random from our large series are shown. The results group closely as shown by the low standard deviation in each case.

DISCUSSION. In the original attempt to apply the inulin and diodrast clearance methods to the rat the importance of a representative plasma sample was recognized. A clearance period of one hour was used and a mid point blood sample obtained. Since one hour had been allowed for equilibriation of the test substance before beginning the clearance period it was felt that the slope of fall of the plasma level during the second hour would be uniform and a mid point sample would accurately represent the mean. While consistent results were

obtained with the method it was admittedly laborious. Dicker and Heller (2) modified the technique to use a short clearance period of 10 to 30 minutes and introduced the device of a terminal blood sample as an estimate of the mean. It was not practical to test the validity of this end point determination since the diodrast analysis required sizable amounts of plasma and precluded the possibility of studying a plasma curve.

More recently Meyer Friedman (3) has studied the clearance of PAH in the rat and arrived at certain interesting conclusions. His method is open to considerable criticism, however, in the light of the present findings. For example, he studied the clearance of 100 mgm. of PAH in the hour following subcutaneous injection. This amount gives a markedly humped plasma curve and is well above the saturation limit. We cannot but feel that the ensuing end point plasma levels, as published, are essentially meaningless.

Dicker and Heller reported no variation in the inulin clearance with urine flow in the range studied. The present observations confirm these findings for low rates of urine formation (up to 0.75 cc./hr.). On the other hand, both Friedman (3) and Braun Menendez and Chiodi (10) have shown an increase in the inulin clearance with higher flows. Estimates derived from Friedman's data indicate that C_{PAH} is actually increased by the excessive hydration, and this increase in renal plasma flow would seem the obvious explanation for the increased glomerular filtration rate observed. It does not seem over-cautious to assume that urine flow rates exceeding 1 cc. per hour involve far reaching changes in renal hemodynamics which invalidate any attempt to interpret the clearance values obtained.

The clearance data of Braun Menendez and Chiodi are especially interesting. Their data are complicated by the presence of hydration since they were interested in this particular feature, but, in general, using a technique similar to that of Dicker and Heller they obtained values for C_{IN} and Tm_D of the same order as those here presented. Similarly, C_D was comparable to the normal value here given for C_{PAH} when urine flow was of similar magnitude and plasma diodrast did not exceed 3.5 mgm. per cent iodine.

In the present series the normal renal plasma flow, C_{PAH}, is higher than that previously reported and the C_{PAH}/Tm_{PAH} ratio higher than in any other species yet studied. It is not unexpected that this should be so when, for example, the normal pulse rate in the rat is approximately 300 per minute (11) when compared with 70 for man.

SUMMARY

1. Renal function has been investigated in the rat using inulin and sodium p-aminohippurate.

2. The calculation of results is most suitably based on surface area. The normal values obtained, expressed per 100 cm² of body surface are as follows: C_{IN} 0.36 $\pm$ 0.04 cc./min.; C_{PAH} 2.31 $\pm$ 0.18 cc./min.; Tm_{PAH} 0.10 $\pm$ 0.01 mgm./min.; F. F. 15.7 per cent $\pm$ 2.3; and C_{PAH}/Tm_{PAH} 22.1 $\pm$ 2.0.

3. Renal plasma flow per unit of tubular excretory tissue is greater than in other animals investigated and may be related to hemodynamic differences.

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RECOVERY OF A PRESSOR PRINCIPLE FROM THE BLOOD PLASMA OF CATS GIVEN KIDNEY EXTRACTS

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In a previous communication (1) a pressor principle was described which was found to appear in the blood plasma of cats that had died of several causes or that had been subjected to a period of hemorrhagic hypotension. The pressor principle was found in the plasmas of cats in which the kidneys remained intact but not in the plasmas of cats which had previously been nephrectomized. This pressor substance appeared to be characterized by its ability to cause a sustained elevation of blood pressure (BP) when injected intravenously into cats nephrectomized 1 to 2 days before, and contrariwise, the elevation in BP was slight and not sustained when the same material was injected into normal non-nephrectomized cats. These findings suggest that the kidneys are concerned in the production of this pressor principle and that they may also be the source of its inhibitor or antagonist.

It was also reported (1) that when kidney extract containing renin was injected into nephrectomized cats the resulting pressor response often did not subside completely, a sustained elevation of BP 10–15 mm. Hg above the base level being observed.

This observation suggested the possibility that the kidney extract might contain the sustained pressor principle in addition to renin, and that if larger quantities were administered over a longer period of time a substantial quantity of the pressor principle might be recovered from the cats' blood. Such a finding would offer further support for the renal origin of the principle.

METHODS. The kidney extracts were prepared from kidneys removed from normal cats under anesthesia. One hundred grams of whole kidneys were stirred in a Waring Blendor with 300 ml. of 2 per cent sodium chloride solution, after which glacial acetic acid was added to a concentration of 2 per cent. The insoluble material was removed by centrifugation and discarded. To the supernatant fluid ammonium sulfate solution was added to 0.6 saturation. The resulting precipitate was collected on Buchner funnels, suspended in water, and dialyzed 16 hours or until free of ammonium sulfate. The supernatant fluid from the material remaining in the sac was used as the kidney extract.

The extracts were injected intravenously into 3 series of anesthetized cats: 1, unoperated normal animals; 2, those bilaterally nephrectomized approximately one-half hour before, and 3, those nephrectomized 2 days before. The extracts (representing 13–34 grams of original kidney) were given in repeated doses over a period of 1 to 2 hours and, after varying amounts of time following the last injection, the cats were bled using heparin as the anticoagulant.

The plasmas obtained were assayed for their ability to cause a sustained pressor response. For this test, 2 ml. quantities were injected intravenously into pithed cats, the kidneys of which had been removed 2 days before. The technic for preparing the animals has been described elsewhere (1). With one exception each plasma was tested on two or more cat preparations and its activity evaluated by measuring the sustained elevation in BP in millimeters of mercury (see legend, fig. 1).

Results. In figure 1 is presented a summary of the pressor assays of the cat plasmas. Those from the "2-day nephrectomized" cats were, in general, quite

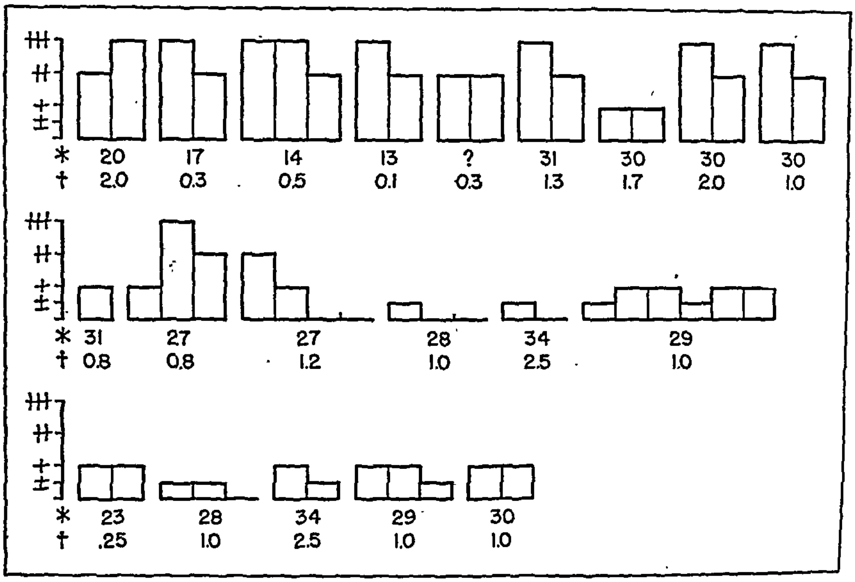

Fig. 1. Graphs showing sustained pressor activity of plasmas recovered from 3 groups of anesthetized cats given cat kidney extract intravenously; upper row, "2-day nephrectomized"; middle row, acutely nephrectomized; bottom row, non-nephrectomized. *—number of grams of original cat kidney from which the kidney extract injected was derived. †—number of hours elapsed after last kidney extract injection before cat was bled to obtain plasma. Each bar represents an individual test in a separate pithed cat. Ordinate scales indicate magnitude of sustained pressure elevation. ± 5–10 mm. Hg; + 10–25 mm. Hg; ++ 25–45 mm. Hg; +++ 45–65 mm. Hg; ++++ 65 mm. Hg or greater.

active and the sustained pressor responses compared in magnitude with those obtained with the injection of hemorrhagic shock plasmas (1). The plasmas from both the non-nephrectomized and the acutely nephrectomized cats usually showed distinctly less activity with only minimal or 1-plus sustained pressor responses. The plasmas from the acutely nephrectomized animals did not exhibit significantly greater sustained pressor activity than those from the non-nephrectomized cats.

Since large quantities of renin were present in the kidney extracts administered to the cats, the amounts of renin remaining in the plasmas were determined.

The present experiments suggest that the sustained pressor principle may be a new and separate entity, physiologically distinct from renin, but extractable from kidney tissue along with renin.

We wish to acknowledge the technical assistance of Robert M. Sanders, William R. Cherry and Ora A. Harvey.

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THE CAROTID-MANDIBULAR REFLEX IN ACUTE RESPIRATORY FAILURE[1]

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Gasping in the isolated ischemic rat head has been used to determine the influence of drugs and of other factors on survival of this primitive respiratory mechanism during asphyxia (1–5). The advantages of this simple technique, which requires little time and less equipment for the study of various phases of respiratory center activity, are obvious. The purpose of these investigations is to examine the neurological basis of the mandibular respiratory movement, to which the meaning of the term "gasp" will be restricted, and to analyse the effects of oxygen lack and carbon dioxide excess.

METHODS AND RESULTS. *Discharge time.* Selle has shown that the survival of gasping is inversely proportional to age for animals, up to or slightly past the weaning period. A graph of this function for the animals in our colony was established (fig. 1) as a base of reference. The method and apparatus used for decapitation were similar to those described previously (4). Since there is no sex variation within any given age group, both male and female rats, of the Wistar strain, were used. They received no special treatment prior to decapitation. All measurements are given in terms of "discharge time," the interval between the first and last gasps. This measurement was used rather than survival time, measured from the time of decapitation, because it represents a unit of respiratory response, which indicates a certain minimum activity of the respiratory center. It is comparable in procedures which induce gasping after widely varied times, such as decapitation and nitrogen inhalation.

It has been shown that, in young animals particularly, two series of gasps, separated by a period of apnea, occur (1, 4). The use of substances preventing oxidative or anaerobic reactions has shown that the first series of gasps depends on aerobic mechanisms, the second on anaerobic ones.

Figure 1 represents a total of 220 animals evenly distributed throughout the age range. The curve showing discharge time also represents, of course, the end of the second series. The initial period of apnea following decapitation averages 4 seconds (3.2–5.0); the first series of gasps averages 3 seconds (0.5–6.7) and 4.5 gasps (1–8); and the period of apnea between the two gasp series averages 16 seconds (13.5–20.0). All these values are constant throughout the age range. In contrast, the second series of gasps decreases in duration and number, which vary together, by over 25-fold between one and five weeks of age; and thus controls variation in discharge time. A separate brain enzyme system promoting

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anaerobic oxidations is probably involved in the second series. Its value to the relatively hypoxic fetal organism is obvious.

Reflex nature. In a preliminary report (6) it was shown that only when the carotid bodies remain connected to the brain does gasping occur. This was established for the decapitate rat and the dog with clamped trachea. To further

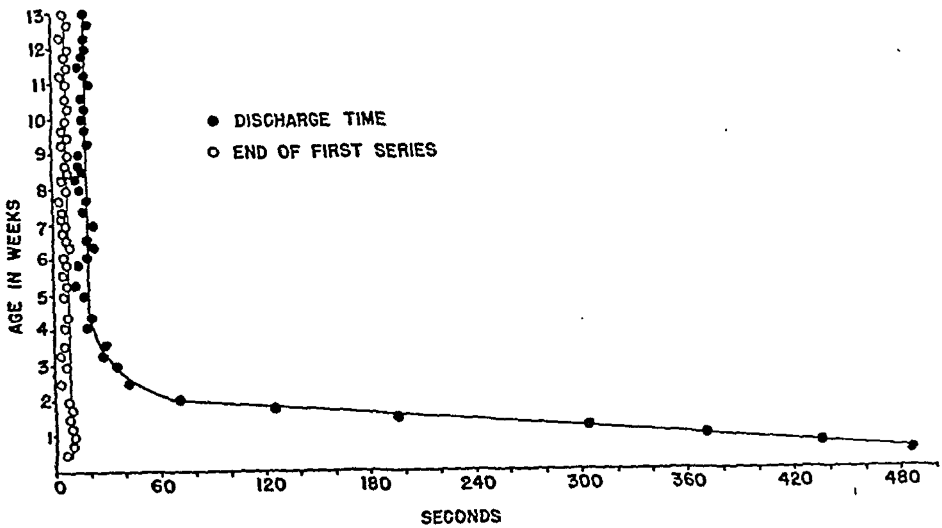

Fig. 1. Showing that variation of mandibular time with age in the untreated isolated ra head is entirely a function of the second anaerobic series of gasps. Each pair of points represents the mean value from five rats of the same age. Discharge time is measured from the first gasp; not from the point of decapitation.

TABLE 1

Showing the necessity of the carotid reflex mechanism in gasping produced by tracheal occlusion or cyanide poisoning

Rats were four weeks old. Bracketed figures give range.

	GASPING PRODUCED BY TRACHEAL OCCLUSION			GASPING PRODUCED BY CYANIDE POISONING		
	Number of rats	Average discharge time	Average number of gasps	Number of rats	Average discharge time	Average number of gasps
		sec.			*sec.*	
Carotid mechanism intact...............	10	138 (126–144)	20 (17–24)	10	851 (709–883)	153 (141–163)
Carotid mechanism denervated..........	10	0	0	10	0	0

substantiate the reflex nature of gasping, when produced by procedures other than decapitation, tracheal occlusion was performed on rats with denervated carotid bodies and the discharge times recorded. Four-week old rats were operated under chloralose, which has been shown to potentiate carotid reflexes and increase discharge time (6). In ten rats, denervation was completed; in ten similar controls a dummy operation was performed. As shown in table 1, only

the control group showed gasping. The denervated animals developed hyperpneic respiratory movements after occlusion of the trachea, but at no time did mandibular movements accompany these efforts.

In a similar series, gasping due to cyanide poisoning was also shown to be entirely a carotid reflex response. All animals given potassium cyanide (10 mgm. per kilo i.p.) died, but only those with innervated carotid bodies gasped (table 1).

The respiratory center. Having established the character of the carotid-mandibular respiratory reflex, the question arises as to which link in this reflex arc is the first to fail during anoxia and asphyxia. Since the carotid body chemoreceptors continue to discharge maximally for 20 to 30 minutes after death (7), one may safely assume that gasping does not cease through failure of these elements. That the efferent limb of the arc is similarly still functional when gasping ceases, was shown by testing the linguo-mandibular reflex. This also involves the motor muscles and peripheral connections of nerves V and VII, and could be elicited for a short time after gasping had ceased. Possible current leakage from the stimuli applied to the tongue was excluded by careful insulation. The respiratory center, then, must be the weakest link in the reflex chain, and discharge time is a measure of survival of that aspect of the respiratory center concerned with the gasping reflex.

Control of gasping. In the decapitate preparation, the tension of oxygen in the respiratory center and carotid bodies begins to fall, and of carbon dioxide to rise, at once. The rôles of hypoxia and hypercapnia in gasping and the locus of action were determined as follows.

A two-liter glass chamber was filled with a desired N_2-CO_2 mixture by water displacement, an 11-day old-rat introduced, and the system continuously flushed with the water-saturated gas mixture at 800 ml. per minute. Twenty animals were run at each of eight atmospheres. After placing an animal in nitrogen, a period of violent struggling began within twenty seconds and lasted for about fifteen. Near the end of this time, an intense apneic gasping began to accompany each inspiratory effort. The mandibular movements then gradually became less marked until they were no longer visible (discharge time endpoint), although contraction of the diaphragm and intercostal muscles continued for a short while. The average discharge times are shown in figure 3. In 100 per cent nitrogen this is 142 seconds, 58 seconds less than that after decapitation. (Controls of same strain, age, diet, season.) With increasing CO_2, to 35 per cent, anoxic discharge time increases, to a maximum of 320 seconds, and then declines again. Discharge time varies directly with the total number of gasps, the intervals between gasps being essentially constant.

It is concluded that anoxia alone can promote respiratory activity at the gasping level, but that maintenance of gasping is enhanced by hypercapnia, as in true asphyxia.

The gasping pattern elicited by exposure to anoxic and hypercapnic atmospheres indicates a continuous and constant level of discharge, with no grouping into the two separate series of the young decapitate preparation.

Nevertheless, iodoacetate (1.0 mgm. i.p., 15 min. before anoxia, 45 rats) cut the average discharge time to 20 to 30 seconds, regardless of carbon dioxide concentration. Since iodoacetate eliminates the second series of gasps in the decapitate preparation by interference primarily with the anaerobic oxidative mechanisms (4), it would seem that in the simple anoxic preparation, also, aerobic processes support the initial gasps, and anaerobic ones the later gasps. And, as in the variation of discharge time with age, the differences in discharge time with changing carbon dioxide tension reflect only the duration of the anaerobic series of gasps.

The effect of hypercapnia without anoxia was tested by placing similar rats in atmospheres of carbon dioxide and oxygen. With increasing carbon dioxide

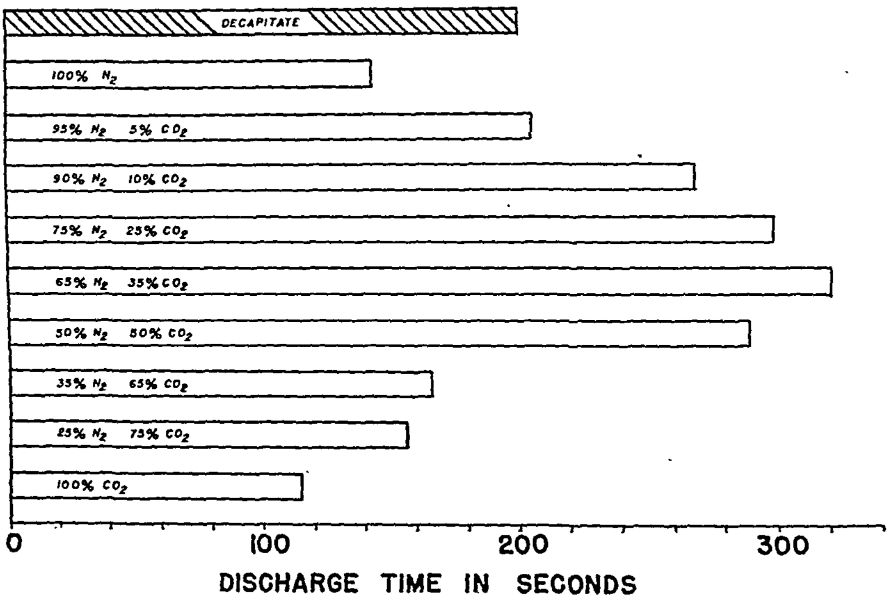

Fig. 2. Showing the effect of various carbon dioxide concentrations on discharge time of untreated 11-day old rats placed in O_2-free atmospheres. Each bar represents the mean value from 20 animals placed in the indicated N_2-CO_2 mixture. Lowest t value is 30. The shaded bar indicates discharge time for the comparable isolated head preparation.

concentration, up to 35 per cent, no gasps were evoked, even on prolonged exposure. Higher concentrations of carbon dioxide produced erratic gasping patterns and death. Since concentrations of this magnitude markedly decrease the oxygenation of the blood and interfere with tissue oxidations, a factor of hypoxia cannot be eliminated. It is obvious that hypercapnia alone has little ability to produce gasping. Anoxia supplies the main drive and CO_2 concentration modulates it.

To determine the site of action on the gasping reflex, a preparation was devised whereby the chemical environment of the carotid chemoreceptors and that of the respiratory center could be varied independently. This involved perfusing the carotid bodies, isolated from the main vascular system, with the circulation from another animal, similar to the method of Heymans, et al. (8). Two

male rats were anesthetized with chloralose. Both carotid forks of one were isolated and perfused by blood from and returning to the carotid arteries of the second (fig. 3). Since the respiratory center is chiefly supplied by the vertebral and spinal arteries, ligation of the common carotid arteries of rat 1 caused little impairment of medullary circulation. Heparin (0.02 gram per kilo i.v.) was administered to rat 2 at the end of the operation.

Rat 2 functions as a perfusion pump and also, since its own carotid bodies are intact and receiving their usual blood supply, as an indicator. When it gasps, the blood perfusing the carotid bodies of rat 1, as well as its own, is obviously

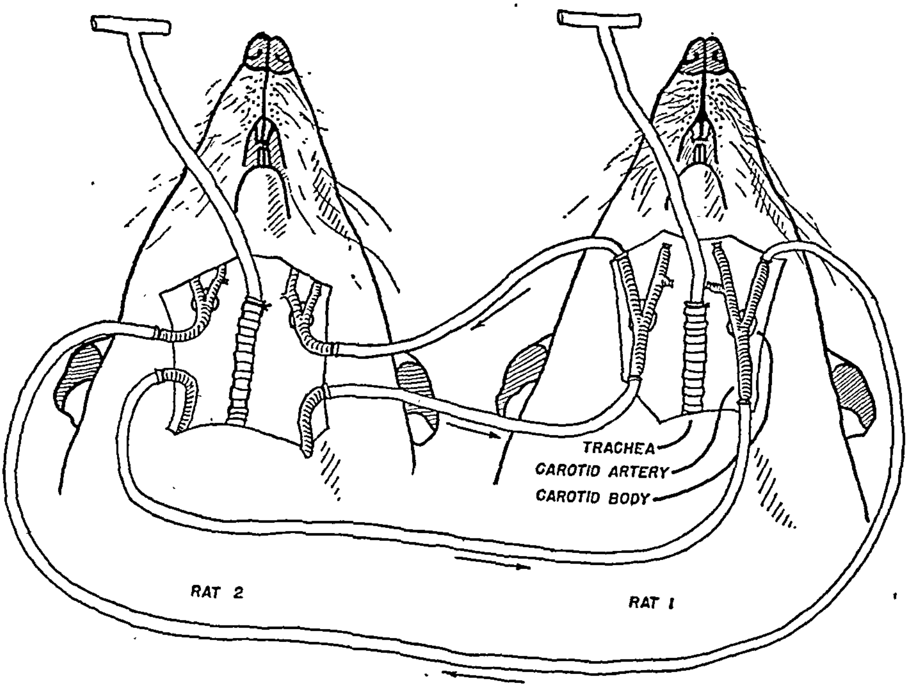

Fig. 3. Diagram of the method used to perfuse the carotid body of one rat (white male 200 grams) with blood from a similar animal. The common and internal carotid arteries were cannulated with polished hypodermic needles and rubber tubing was used for connections. Rat 2 is heparinized to prevent coagulation. The T-tube tracheal *cannulae* lead to valves by which different atmospheres can be administered.

capable of initiating the gasping reflex. A desired gas mixture was administered to each animal from a rubber bag attached to a T-tube in the trachea with appropriate one-way valves.

The results of such experiments are summarized in table 2. It is clear that CO_2 stimulation of the carotid body cannot initiate gasping unless the neural centers are anoxic. Conversely, with an anoxic center, the full potentiation by CO_2 can be obtained when this acts only on the carotid system. Thus, in the simple inhalation experiments, 35 per cent CO_2 increased discharge time 129 per cent; and, in the perfusion experiments, 35 per cent CO_2, acting only on the carotid body, increased it 141 per cent.

DISCUSSION. Although gasping has little or no function in normal respiration of the mammal, it nevertheless provides an index of neurone activity. As such, and because of its unique ability to distinguish functionally between glycolytic and aerobic oxidations, the technique described may prove useful in investigating the metabolism of the respiratory center. Although the animal is *in extremis* when gasping is observed, the conditions are probably still more physiologic than with in vitro methods.

An analysis of gasping is of value not only in relation to the use of this phenomenon as an indicator of metabolic activity, but also in relation to respiratory control. Respiratory movements of fish consist of little more than gasps, indicating the primitiveness of this response. Lumsden has described (9)

TABLE 2

Showing the effect of perfusing the carotid bodies of rat 1 with blood from rat 2 when each animal is inhaling a different atmosphere

Gasping of rat 2, when it inhaled N_2 or N_2-CO_2, indicates that the carotid bodies of rat 1 were sufficiently stimulated to evoke gasping. N_2 = 100% N_2; N_2-CO_2 = 65% N_2-35% CO_2. N_2-CO_2 is referred to as asphyxic. Four experiments were done at each inhalation pattern. Bracketed figures give the range of the four results.

INHALED GAS		CONDITION OF RAT 1		RESPONSE OF RAT 1	
Rat 1	Rat 2	Carotid body	Respiratory center	Number of gasps	Discharge time
					sec.
Air	N_2	Anoxic	Normal	0	
Air	N_2-CO_2	Asphyxic	Normal	0	
N_2	Air	Normal	Anoxic	0	
N_2-CO_2	Air	Normal	Asphyxic	0	
N_2	N_2	Anoxic	Anoxic	5 (4–7)	16.5 (14–19)
N_2-CO_2	N_2	Anoxic	Asphyxic	6 (5–7)	15.0 (12–18)
N_2	N_2-CO_2	Asphyxic	Anoxic	12 (10–14)	40 (35–42)

a primitive gasping center located in the medulla and unresponsive to changes in carbon dioxide tension. It is possible that the carotid-mandibular reflex measures activity of this center, released as more recent centers succumb to anoxia in a reverse phylogenetic order (10). The alternative hypothesis that anoxia stimulates the central mechanism would seem to be contraindicated by the body of evidence against direct anoxic excitation of the respiratory center (11).

Two factors are necessary for the appearance of the carotid-mandibular reflex; anoxia of the respiratory centers, and stimulation of the carotid chemoreceptors. Potentiation of the response by carbon dioxide acting on the carotid end organs is probably due merely to stronger afferent bombardment of the medullary centers.

Preliminary work indicates that the variation of response time with age is a function of the central neurones and not of the carotid body.

SUMMARY

Gasping of the severed rat head has been used by several workers as an index of activity of medullary respiratory centers. Present experiments establish the reflex character of gasping in several types of anoxia and locate the actions of anoxia and hypercapnia in the reflex arc.

After decapitation, cyanide administration, or inhalation of oxygen-free gas mixtures, gasping occurs when the carotid bodies remain functionally connected with the medulla but is entirely absent when they are disconnected. Gasping is terminated by central failure, for the carotid receptors discharge long after gasping ends, and the lingual-maxillary reflex (engaging the same efferent arc) also outlasts it.

Discharge time (from first to last gasps) decreases with age. For 11-day rats inhaling pure nitrogen, it averages 142 seconds, as compared with 200 after decapitation. Addition of CO_2 to the N_2 (but not to O_2) increases discharge time, to a maximum 320 seconds at 35 per cent CO_2. That the prolongation depends on anaerobic processes is shown by the ability of iodoacetate to cut discharge time to 25 seconds, whatever the CO_2 concentration.

By "perfusing" the carotid bodies of one rat with the blood of a second and varying independently the gas inhaled by each, the medullary centers and the carotid bodies of an animal were exposed to different conditions. Anoxia both of the brain and the carotid body must be present for gasping to occur. The potentiating action of CO_2 is exclusively on the carotid body.

These findings, while establishing important peripheral factors in the gasp response, do not vitiate its use in investigating the action of central neurones.

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ture on the skin surface rose quickly to temperatures considerably in excess of those normally occurring even in hot environments (range from 39 to 45°C). Since the couples were necessarily placed on the surface, however, the high temperature was attained here somewhat before the gland cells themselves were heated at some distance below the skin surface. This factor alone may explain in large part the delay in sweating responses behind the rise in surface temperature.

Following this delay a few very large, dark sweat spots appear in the area of highest temperature with little or no sweating on adjacent areas. Sometimes smaller sweat spots may be noted briefly on adjacent areas (fig. 1) but as long as the temperature of these areas remains low, and the local heating is not excessively painful, sweating does not persist except for the normal cyclic periods of activity. Sweating increases in intensity in the locally heated area and is marked by increasing numbers of large droplets (which appear on the record as large, dark spots, indicating excessive output by the individual glands). Cyclic activity in this area is depressed or obscured by the profuse sweating and many glands appear to be continuously active for the duration of the exposure to heat. If excessive heating is prolonged, 70 to 80 per cent of functional sweat glands in the area may be stimulated, but it was usually observed that the primary response to direct local heating was an increased output of individual glands rather than stimulation of a maximum number of glands.

When the heat was turned off both the size and number of sweat spots immediately decreased as local sweating declined more or less progressively with the fall in surface temperature. Sweating then disappeared when the skin temperature was still a few degrees above the starting normal. It may be suggested therefore that a critical temperature exists above which direct, locally induced sweating occurs and below which it does not occur. This critical temperature is several degrees above that necessary to induce sweating by reflex, when heat is applied to a larger surface area.

Figure 2 demonstrates this local sweating response to concentrated heat on the forearm. Note the relationship between sweating and local surface temperature. The fact that sweating is present at lower temperatures as the area cools compared with the period of heating is probably related to the insulation and heat storage capacities of the skin. In one experiment the subject complained of very severe pain during the heating period and sweating appeared diffusely spread over the heated and adjacent non-heated areas alike, simultaneously with the development of the severe pain. The rapidity of onset of this sweating together with the smaller and more discrete character of the sweat spots identified this response as purely reflex in nature, and contrasted with the delayed appearance of large sweat spots confined to the heated area.

In a few experiments it was noticed that after prolonged exposures to such local heat (at temperatures well above 40°C) sweating continued for as long as several hours in the central area of highest temperature. Such sweating continued in spite of the fact that skin temperatures returned to normal. In some experiments, the temperatures of these local areas were reduced several degrees

below the starting normal temperatures by application of ice-filled containers, without abolition of the persistent local sweating. In all such experiments, damage to the skin was evidenced later by the appearance of local edema and blister formation. In fact, after some experience the experimenter could predict

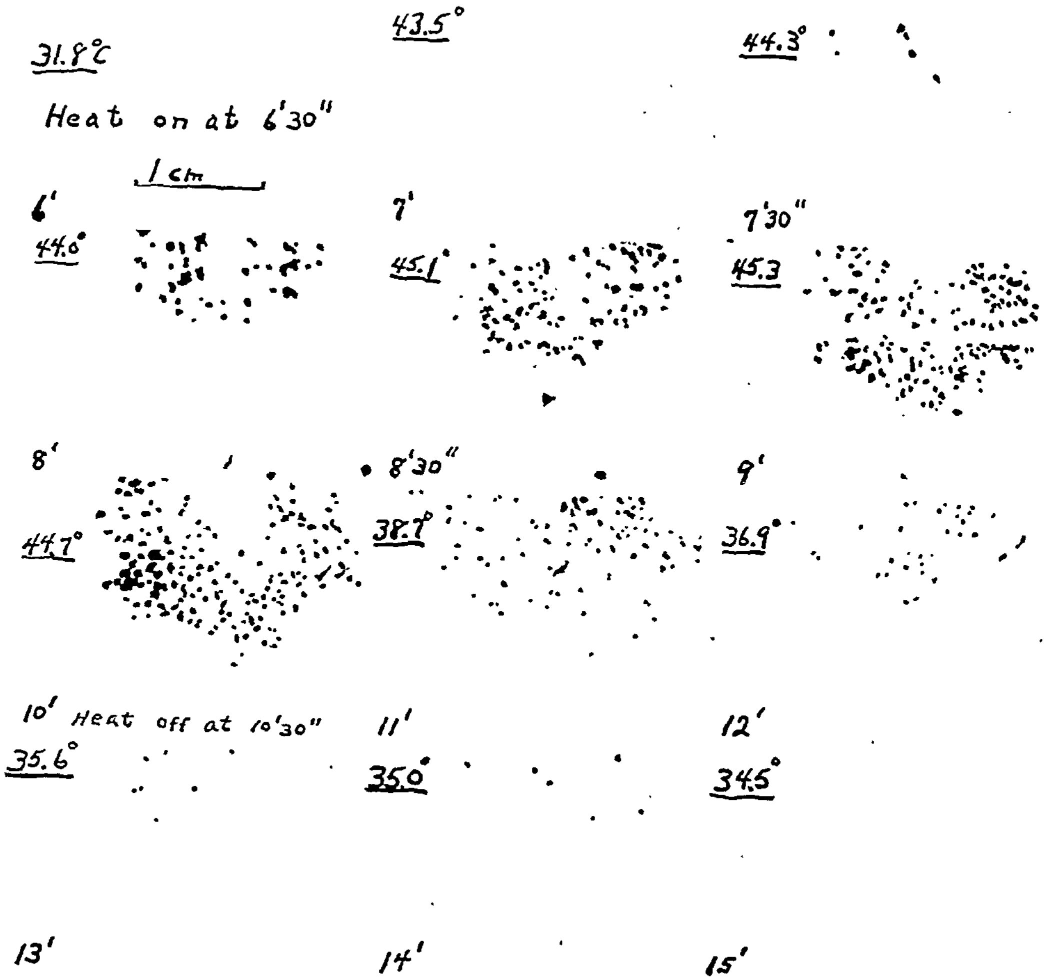

Fig. 2. Experiments demonstrating sharply localized response to local radiant heat on forearm. Records of 20 second duration taken at 30 second intervals. No sweating was present during first five minutes of control period, and successive records starting at sixth minute show development and regression of individual gland responses (individual sweat spots) during and following heat application. Enlarged photograph of original records retouched for purposes of reproduction. Skin surface temperatures of heated area shown in upper left corner of each record.

skin damage by excessive heat, even before blister formation was evident, by the persistence of local sweating.

Kuno (1934) reported that the irritability of the "sweat mechanism" including the centers in the central nervous system varies seasonally, probably involving processes of acclimatization. To our knowledge, however, there is no

evidence that such changes in critical temperatures of individual glands to direct effects of heat occur. Table 1 summarizes the protocols of a number of experiments in which the skin temperature was rapidly elevated until local sweating was established. Since the temperatures were recorded from the skin surface rather than at the precise depth of the sweat glands, it is not advisable to consider the values as true threshold temperatures, and as indicated above, threshold values appear to lie at somewhat lower levels. The data do indicate however that such threshold levels of stimulation do exist. This is demonstrated clearly in subject Smi (2/7/47) where in successive experiments sweating failed to appear when the surface temperature was maintained at 41.15 to 41.85°C for

TABLE 1

SUBJECT	DATE	STARTING SKIN TEMPERATURE	TIME TO INDUCE FIRST LOCAL SWEATING	SKIN TEMPERATURE AT FIRST LOCAL SWEATING	
		°C			
Slo	5/3/46	34.25	1 min. 40 sec.	42.6	—Room temperature 23–30° in
Slo	5/6/46	33.10	1 min.	42.2	all summer experiments
Slo	5/7/46	33.30	2 min.	41.0	
Ra	5/4/46	33.15	1 min. 30 sec.	43.2	
Ra	5/3/46	32.9	2 min.	42.6	
Ra	5/18/46	32.2	3 min. 30 sec.	39.6	
Ra	5/22/46	32.0	1 min. 30 sec.	45.5	
Ra	5/20/46	31.5	1 min. 30 sec.	43.7	
Ra	5/20/46	32.05	2 min.	38.4	
Ra	6/22/46	34.1	2 min. 30 sec.	45.35	
Ra	6/6/46	33.8	6 min. 30 sec.	42.9	—Temperature raised slowly
Hal	5/23/46	33.9	8 min. 30 sec.	45.5	—Temperature raised slowly
Ri	5/20/46	31.2	2 min.	43.8	Response delayed several
Ri	5/20/46	32.7	2 min. 30 sec.	44.7	minutes after maximum
Ri	5/20/46	33.4	2 min.	44.7	temperature had been attained
Smi	2/7/47	30.2	No sweating	41.85(maximum)	16.0—Room
Smi	2/7/47	31.2	2 min. 40 sec.	42.7	17.0 Temp.
Ra	2/7/47	30.0	1 min. 40 sec.	44.2	17.0
Ra	2/7/47	30.1	1 min. 30 sec.	41.4	16.0
Ri	2/25/47	31.7	1 min. 30 sec.	44.8	25.5
Dee	2/24/47	32.0	1 min.	44.3	21.0

6 minutes, but sweating did occur in a subsequent experiment as soon as the surface temperature reached 42.7°C.

Considerable variation in temperatures at which sweating may first occur, even in the same individual at different times, is indicated. These variations possibly may be referable to differences in temperature at the precise level of the contractile elements in the sweat glands.

Thus it is not possible to say that small changes in the threshold temperature at which sweat glands respond to direct stimulation do not exist in the general acclimatization of the sweating mechanism from summer to winter, but it appears from table 1 that if such changes do occur they are relatively small. It is

likewise impossible to explain relatively large differences in so-called thresholds (such as are apparent in subject Ra) at different times. It is evident however that the range of such variations are similar in the summer and winter experiments. If temperatures are raised more slowly sweating may occur at a somewhat lower temperature, but this is probably directly related to the temperature gradients induced and even in these experiments a threshold temperature must be reached before sweating occurs.

Evidence thus far presented indicates that the sweat glands will respond to directly applied heat, but the possibility of a sharply localized reflex arc is not completely ruled out. From the diffuse type of response observed in experimentally demonstrated reflex sweating (Randall, 1947) it is difficult to reconcile such sharply localized sweating (with complete absence of sweating in adjacent areas in some experiments) to anything but a direct response of the glands.

To check this, normal sweating was abolished in a small area (about 15 mm. diameter) by iontophoresis of atropine (pad soaked in 10 cc. of solution containing 1 mgm atropine, 2 ma for 2 to 5 min.) while sweating continued in all adjacent areas. Application of heat to the opposite arm caused excessive sweating on the normal, adjacent areas but no sweating in the atropinized area. Heat was then applied directly to the atropinized area. Although in no instance did sweating appear as pronounced in the atropinized area as in the adjacent tissue, a large number of glands were activated at about the same surface temperature as shown to be required to produce local sweating. Thus although reflex impulses were blocked entirely by atropine, some sweat glands in the area responded to direct application of heat. Similar results were observed when normal reflex sweating was abolished by intradermal injection of 2 per cent procaine, that is application of intense radiant heat to the wheal resulted in sweat responses.

Finally, the local sweating response to heat may remind one of the local response to faradic stimulation reported by Wilkins, Newman and Doupe (1938) and by Bickford (1938). These workers suggest that the local response to faradic stimulation represents an axon reflex mediated through postganglionic sympathetic fibers. It seems doubtful, however, that local heating as employed in the present experiments could act as a direct stimulant comparable to faradic stimulation. Further, the local sweating response to intense heat was not abolished (although it was decreased) by atropine and procaine. Such observations made on human skin agree with those of Saito (quoted by Kuno, 1934) who obtained sweating by warming the pads of the cat's foot even though the innervation had been sectioned three to six weeks before.

These observations would indicate therefore, that the profuse response of sweat glands to local radiant heat of high intensity is a direct one. The threshold temperatures at which this direct stimulation occurs is considerably higher however than those encountered under normal circumstances.

SUMMARY

Using a method designed to investigate activity of individual sweat glands, the sweating responses to locally applied radiant heat were studied. When

the temperature at the skin surface was rapidly elevated to high levels (39 to 45°C), large dark sweat spots appeared in the heated area, indicating excessive sweat responses by the individual glands. These spots contrasted sharply in size with those induced by normal reflex stimulation. A definite threshold temperature appears to exist, above which a direct response to local heat may occur and below which it does not occur. This threshold appears to lie within a rather wide range of temperatures and the range of variation does not appear significantly different in summer and winter. Pharmacological evidence is presented to suggest that the profuse sweating response of individual glands to extreme temperatures is a direct one in contrast to the usual diffuse reflex response.

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treatment 50 to 70 or even a higher percentage was furnished by fat. In some experiments the prethyroid proportion was completely reversed and calories derived from starch reached 80 per cent in the course of thyroxine administration, while before and after treatment 80 per cent was furnished by lard.

DISCUSSION. The thyrotoxic state produced by prolonged administration of thyroxine increased food intake considerably. The fact that this increase might follow the rise in the rate of metabolism after a lag of some days and persisted 5 to 7 days after the sharp decline of O_2-consumption suggests that increased food intake was not directly related to the injection of thyroxine or the resultant rise in the rate of metabolism. Presumably there must be some mechanism

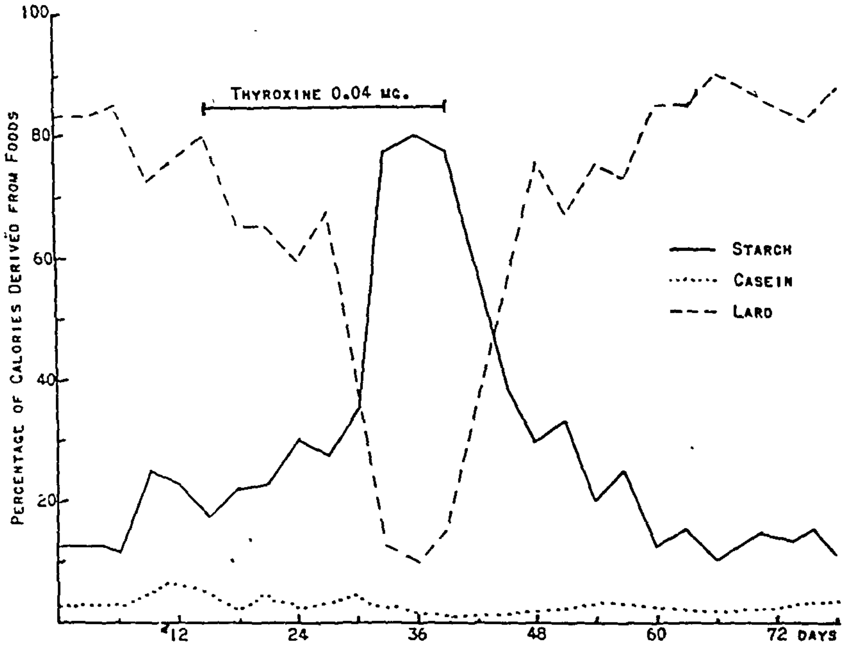

Fig. 4. The effect of thyroxine administration on the proportion of calories derived from different foods.

regulating food intake which is set in motion by the drug directly or as the indirect result of the increase in metabolism.

Experimental evidence presented shows clearly that a very great change in selection was associated with the increase in food intake. During the first days of increased food consumption the qualitative change was less prominent, but later the preference for starchy food became so marked that at the height of the thyroxine effect the additional calories ingested were furnished in many cases almost entirely by carbohydrates. In some cases the consumption of fat fell even below the initial level.

The selective rise in carbohydrate intake is the more remarkable as before and after thyroxine treatment a marked preference for the fatty food was evident. It would seem natural that the necessary additional calories should be

furnished by the food of highest caloric value—at least in an animal having in normal conditions a marked preference for fat.

The fact that prolonged administration of thyroxine is associated with essentially similar—though much more pronounced—changes in food selection as observed in animals exposed to low temperatures (11°C.), may be considered as valuable circumstantial evidence in support of the suggestion advanced in an earlier communication (Donhoffer and Vonotzky, in press) that the selective rise in carbohydrate consumption at low temperature may be due to changes in thyroid function.

Considering the remarkable rise in consumption of carbohydrate-rich food under the conditions described, it seems probable that the composition of food and thyroxine tolerance may be connected. This supposition was suggested by the results of Abderhalden and Wertheimer (1926) and Schlossman (1929) but also by analogy by the observations of Richter and co-workers (1936, 1938), according to which sodium chloride intake of adrenalectomized and calcium intake of parathyroidectomized rats increases considerably. The thesis that an increased ingestion of carbohydrate protects the animal against the damaging effect of long continued thyroxine administration is not supported by the few experiments we have performed to test this hypothesis. Briefly the experiments were as follows. Four groups of 10 mice were fed exclusively diets 1, 2, and 3, or the standard diet, and given thyroxine until death occurred. All showed the same rise in basal metabolism and no significant difference in death rate was evident. Therefore no therapeutic inferences can be drawn from these experiments on the value of dietetic measures in hyperthyroidism.

SUMMARY

Prolonged administration of thyroxine considerably increased the food intake of white mice. The increase in food intake followed the rise of O_2-consumption after a lag of some days and persisted for a similar period after the fall in the rate of metabolism.

The additional calories ingested during the effect of thyroxine were furnished, free selection provided, mainly, in many cases entirely, by consumption of starchy food. At the height of the thyroxine effect this often provided 80 per cent of the calories, while in untreated animals 70 to 80 per cent were furnished by the selection of a fat rich food.

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The above experiments were concluded by April, 1944. War and postwar conditions rendered available literature limited.

THE CLEARANCE OF INULIN AND SODIUM P-AMINOHIPPURATE IN THE RAT[1]

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The natural advantages of the rat as an experimental animal have made it worth while to establish reliable methods for the estimation of renal function in this species. In 1942, two of the present authors presented a method using inulin and diodrast which gave reproducible results and was of value in comparative experiments, although admittedly cumbersome (1). Dicker and Heller, in 1945, criticised the absolute value of the method since ether had been used and renal function probably considerably depressed (2). They presented a modified procedure which was, however, still too cumbersome to be of value for large groups of animals. In both these methods, the emptying of the bladder at the start of the procedure and again ten to thirty minutes later entailed a double hazard, since incomplete drainage at the start or at the end of the clearance period would give abnormally high or low clearance values respectively. Further, no attempt had been made to determine whether plasma concentrations of the test substances measured at the end of the clearance period were valid for the whole of that period.

More recently, Meyer Friedman has estimated the clearance of sodium p-aminohippurate (PAH) in the rat (3), but his methods are open to certain criticisms. It seemed to us that a useful clearance procedure should be established on a firm basis with some absolute validity and adapted for large series of animals. P-aminohippurate, whose advantages are well known (4), was used throughout instead of diodrast.

EXPERIMENTAL. *The relation of dose to plasma level.* Preliminary to arranging a satisfactory clearance procedure, the response of the plasma level to administration of the test substance (inulin or PAH) was investigated. This was considered necessary if the test substance were to be given as a single dose without sustaining infusions and if a single blood sample were to serve as a guide to the plasma level during the test period.

a. *The PAH plasma level after injection.* It was hoped that a quantitative picture of the plasma level could be obtained by following a single subcutaneous administration of PAH. Since clearance is expressed in terms of plasma, while such curves could only be studied using small samples of whole blood from the tail, preliminary experiments were performed to determine whether the whole blood analysis could yield the plasma concentration by calculation. The method of Bratton and Marshall for the analysis of PAH was used (5). Male albino rats were injected subcutaneously with small quantities of PAH and samples of

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blood taken by heart puncture. The hematocrit was determined and a sample of blood analysed directly. The remaining blood was then centrifuged and the supernatant plasma analysed. The precipitated red cells were washed with isotonic saline until the washings were free of PAH. The cells were then laked and analysed for their PAH content. Table 1 shows that the plasma level of PAH can be calculated accurately from the whole blood analysis if the hematocrit value is known.

PAH as the aqueous solution of the sodium salt was then injected into normal rats. The desired doses, 30 to 100 mgm., were prepared by diluting the clinical 20 per cent solution in 2 per cent sodium sulfate so that the required amount was contained in 4 cc. This quantity was injected subcutaneously and successive small samples of tail blood were analysed, 0.1 cc. of blood being used for the determination. Hematocrit values were determined at varying times in each experiment. As a final precaution, the PAH content of the final tail blood sample was compared with that of heart blood obtained at the same time. Curves of this type and in this range were constructed for 14 adult male rats.

TABLE 1

BLOOD PAH A	HEMATOCRIT B	PLASMA PAH		CELL PAH	
		Analysed C	Calculated $A \times [100+(100\text{-}B)]$	Analysed	Calculated $A\text{-}\left[\frac{C\times 100\text{-}B}{100}\right]$
mgm. %	%	*mgm. %*	*mgm. %*	*mgm. %*	*mgm. %*
2.0	46	3.2	3.1	0.2	0.3
1.5	45	2.3	2.3	0.2	0.3
2.0	46	3.2	3.1	0.2	0.3
2.4	48	3.7	3.6	0.2	0.3

As shown in figure 1, the 40 mgm. dose yields a steady plasma level. In this case, the value at 52 minutes was 5.2 mgm. per cent, while the calculated average for that period was 5.3 mgm. per cent. Doses above 40 mgm. cause the plasma level to rise to a maximum within approximately 30 minutes and then to fall. With smaller doses the peak level is reached more rapidly. Apparently, with doses above 40 mgm. the hippurate enters the blood faster than it can be cleared by the kidneys. Thus, where the dose of PAH administered is of a size to produce a pronounced maximum in the curve, the end point plasma value may not represent the mean. Although the plasma concentration falls less rapidly after 60 minutes, clearance determinations based on end point values obtained even beyond this time still deal with a rapidly falling curve. On the other hand, repeated curves obtained from animals given a 40 to 50 mgm. dose showed that a plasma sample taken 50 minutes after injection was representative of conditions existing over the entire period.

These data indicate that the renal saturation level for PAH lies in the neighbourhood of 6 mgm. per cent. No variation in hematocrit was noted despite the repeated small bleedings and there was close agreement between the last tail

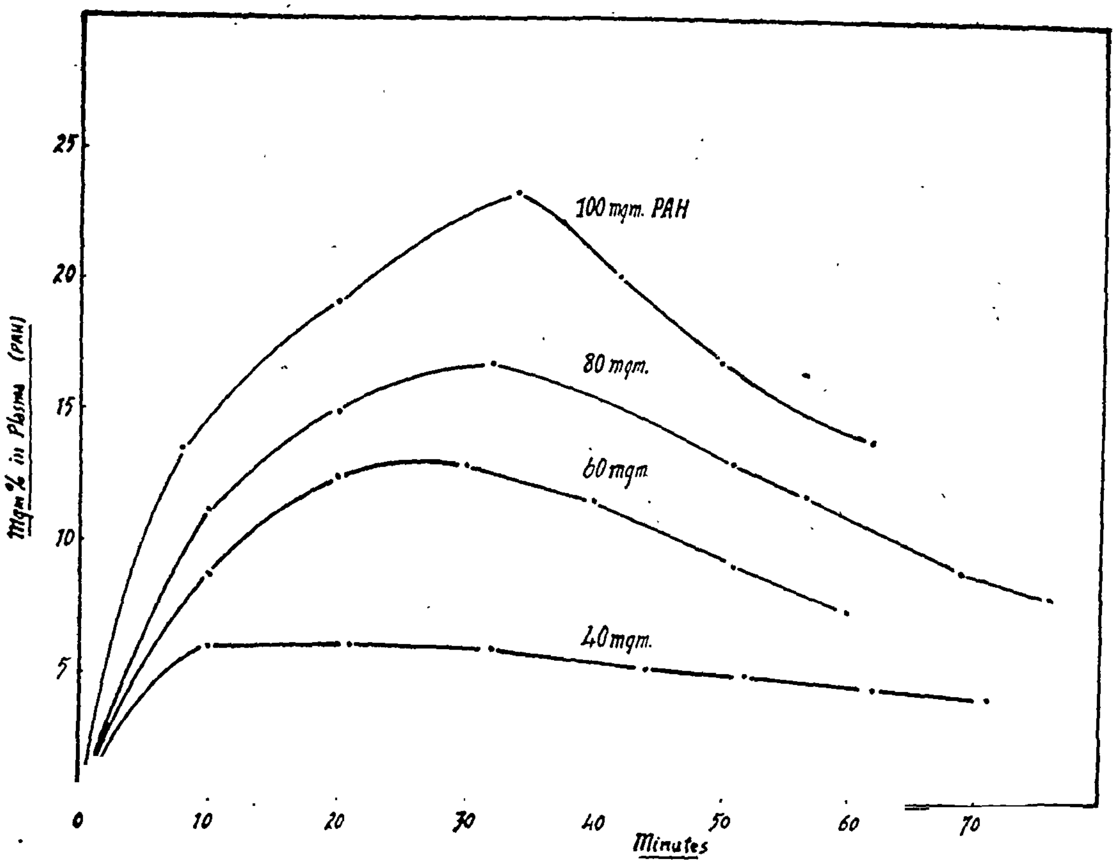

Fig. 1. The relation of the plasma concentration of PAH to the administered dose

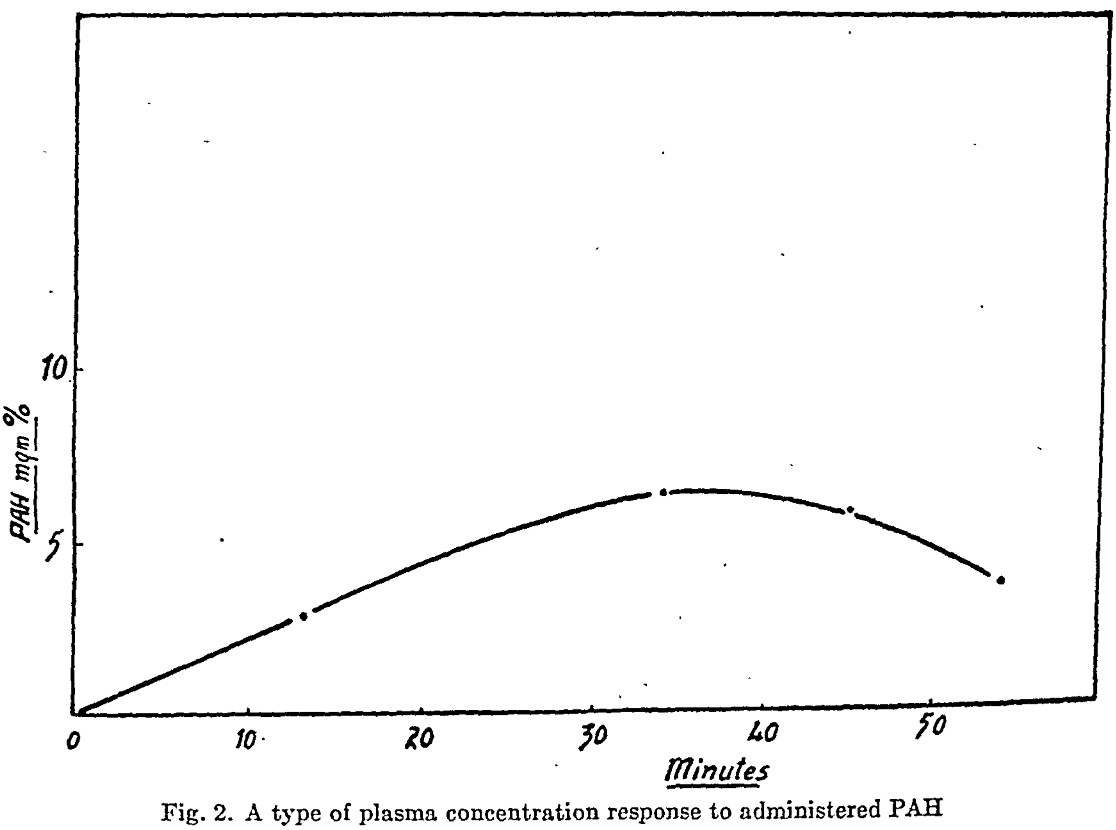

Fig. 2. A type of plasma concentration response to administered PAH

blood analysis and the analysis of the simultaneous heart blood sample. The curve shown in figure 2 indicates that even with low doses, end point plasma values may be misleading. In this animal, the plasma level rose slowly to 6.3 mgm. per cent, then fell rapidly to 3.6 mgm. per cent at 54 minutes, a value not truly representative of the mean. Numerous experiments have shown that when the 50 minute plasma value lies below 4.5 mgm. per cent, the curve may be of this type.

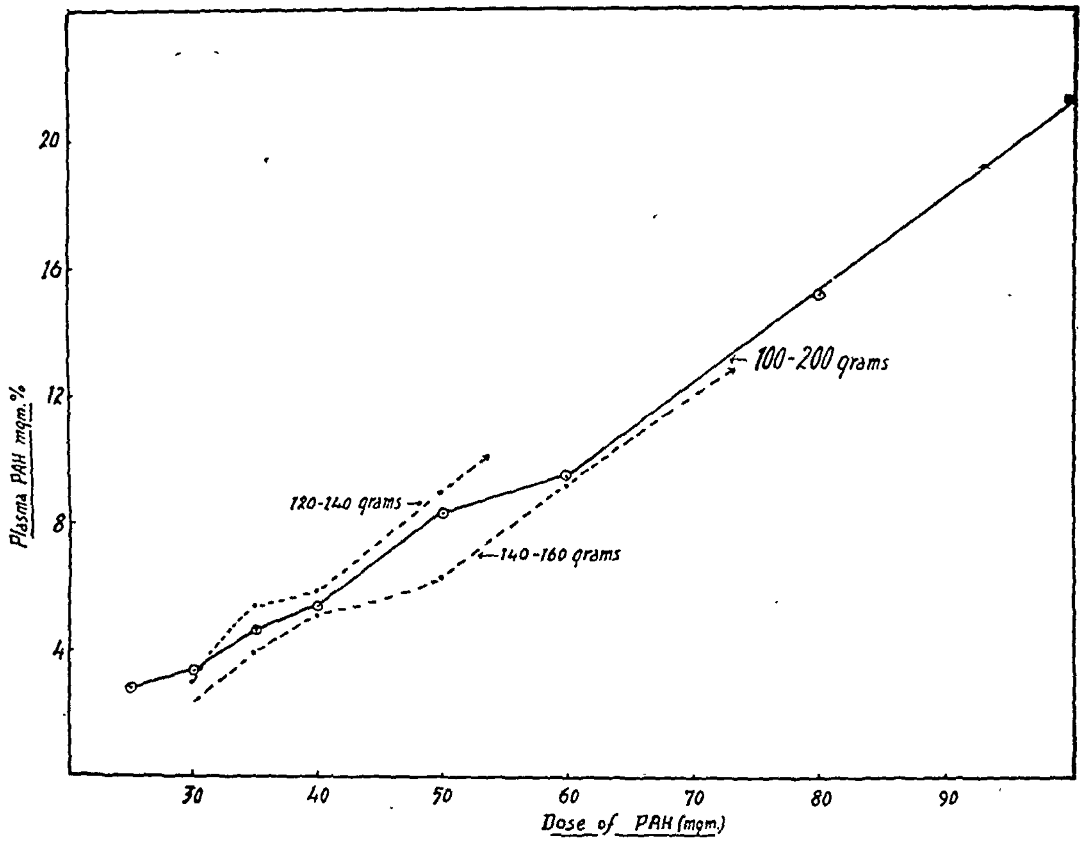

Fig. 3. The relation of plasma concentration 50 minutes after subcutaneous administration of various doses of PAH.

Two conclusions with regard to clearance procedures for PAH using the single dose technique follow from this analysis. 1. If the clearance period begins immediately after injection and lasts 50 minutes, a steady plasma level can be maintained by a suitable dose. 2. If the clearance period starts 30 or 60 minutes after injection, requiring a larger initial dose of PAH, a variably falling plasma level during the clearance period results.

To ascertain the dose of PAH necessary to yield correct plasma levels 50 minutes after injection in different size animals, a large series of determinations was carried out. The data are summarised in figure 3. The plasma concentration 50 minutes after injection is almost linearly proportional to the dose adminis-

tered. The dose is not sharply critical, a difference of 10 mgm. producing a change in plasma level of approximately 2 mgm. per cent. In this composite curve a slight break occurs in the 40–50 mgm. dose range, corresponding to 5–7 mgm. per cent in the plasma. This break, not very apparent in the over all grouping of animals weighing 100 to 200 grams, was very obvious with a more restricted weight grouping. Thus, for animals weighing 120 to 140 grams, and 140 to 160, the curve breaks sharply between 5 and 7 mgm. per cent, a further indication that this is the level of renal saturation.

b. *The inulin plasma level after injection.* As in the case of PAH it was of importance to investigate the variation of the plasma concentration with time after the administration of inulin. This problem was not studied previously because of the lack of suitable chemical procedures. With the publication by Harrison (6) of a method which could measure as little as 2γ of inulin it became feasible to investigate this problem provided that 1, the plasma value could be

TABLE 2

BLOOD INULIN	PLASMA INULIN	
	Calculated	Analysed
mgm. %	*mgm. %*	*mgm. %*
6.5	13.0	14.0
10.0	20.0	20.8
8.0	16.0	15.6
13.5	27.0	26.5

obtained from the whole blood value by calculation, and that 2, in view of the small size of the sample, the necessity for yeast treatment to remove fermentable sugars could be avoided.

It was expected that no inulin would enter the red cell. Thus, assuming a hematocrit of 50 per cent (normal for the rat) the plasma concentration should be twice that of the blood. A series of animals was studied to satisfy this assumption. Inulin was injected, a blood sample taken, and the inulin content of both blood and plasma determined. Table 2 presents typical data, and shows that the whole blood concentration obtained by analysis is directly convertible to the plasma value providing the hematocrit remains unchanged.

In the analysis of whole blood it was found that the inulin content could be estimated without yeast treatment by subtracting a blank blood value obtained from the same animal before the administration of inulin. Indeed, a calibration curve for inulin in blood differed from the aqueous curve only by the blank value of the particular blood used to establish the curve (fig. 4). Investigation of a series of normal animals showed that this blank value was fairly uniform.

A 2 per cent solution of inulin in saline was then prepared, adsorbed on charcoal while hot, and filtered hot through a Seitz EK disc. Three cubic centimeters (60 mgm.) of this inulin solution were injected intraperitoneally to insure uniform and rapid absorption into the blood stream. In our experience inulin

solutions are poorly absorbed from the subcutaneous tissues. The typical plasma concentration curves for a series of animals are shown in figure 5.

From a study of such curves it appeared that the intraperitoneal injection of this amount of inulin yielded a plasma concentration which increased smoothly for at least 50 minutes. The mean value of the inulin concentration in plasma for that period corresponded closely to two-thirds of the value obtained by analysis of a sample taken at 50 minutes.

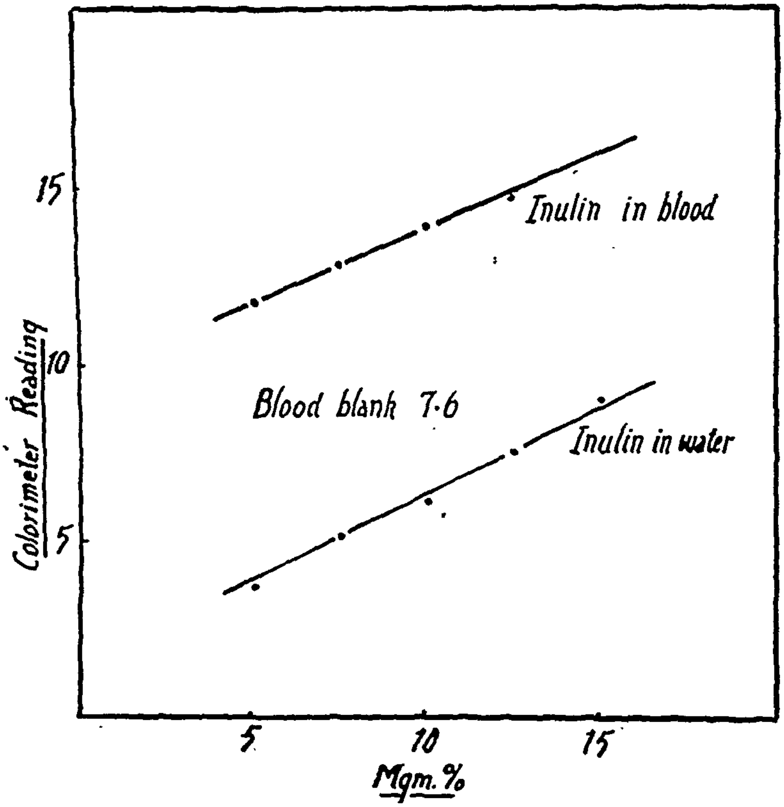

Fig. 4. The colorimetric reading of inulin in blood is the sum of its inulin content plus the blank value for whole blood.

The clearance of inulin and PAH. It became increasingly clear that for the determination of both inulin and PAH clearances, an experimental period extending for 50 minutes from the time of injection was an eminently suitable arrangement. Not only are the plasma concentrations reasonably steady, but in addition, the necessity of draining the bladder at the start of the period is eliminated. This diminishes the hazard of incomplete urine collection. Based on this, a series of clearance determinations were carried out in normal male rats. Although the clearance of inulin and PAH were determined simultaneously, for convenience in discussion they will be separately presented.

a. *The clearance of inulin.* The clearance of inulin, C_{IN}, was investigated in 60 adult rats. The average values obtained were plotted against the plasma

concentration, which ranged from 30 to 60 mgm. per cent. The inulin clearance was independent of plasma level in the range investigated; the average value for C_{IN} when the plasma concentration was 31–40 mgm. per cent was 0.72 cc./100 grams/min., when 41–50 mgm. per cent, 0.75 cc., and when 51–60 mgm. per cent, 0.70 cc. In these experiments urine flow was not excessive, averaging 0.5 to 0.7 cc./hour, and inulin clearance was independent of the rate of urine flow.

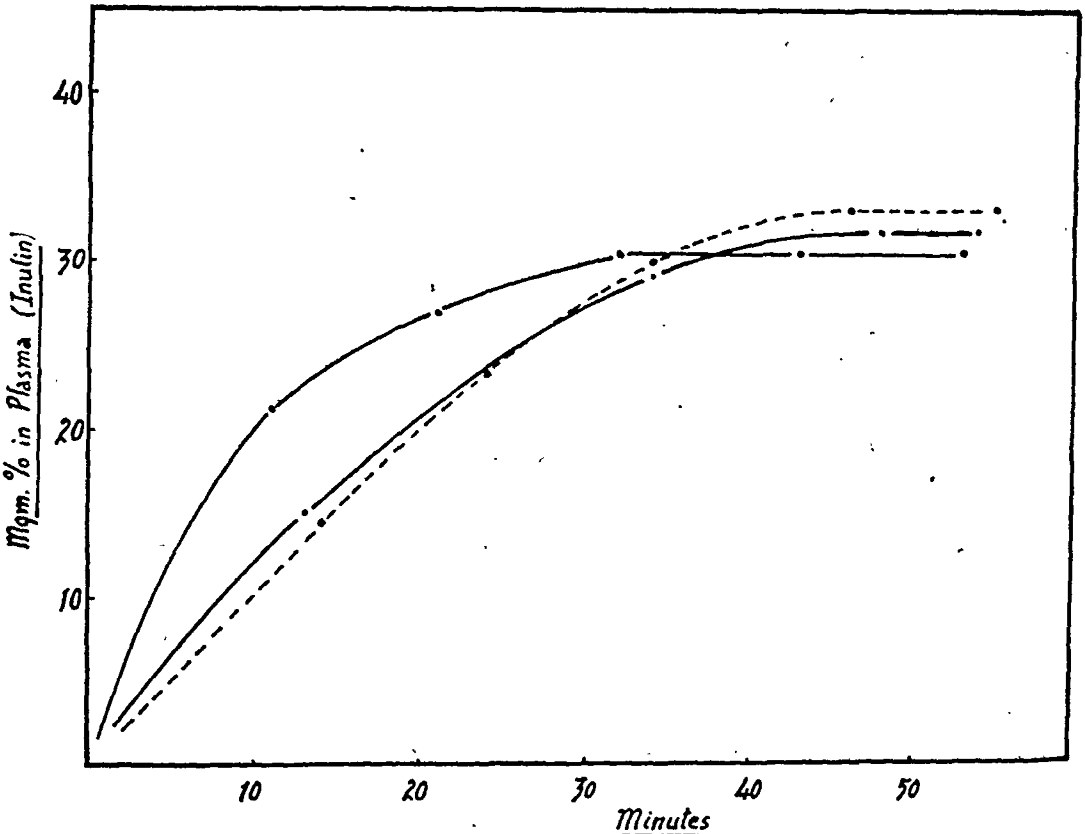

Fig. 5. The plasma concentration of inulin after intraperitoneal administration of an aqueous solution in three animals.

b. *The clearance of PAH.* According to the classical work of Smith and his co-workers (7, 8) the clearance of a substance which is wholly removed from the plasma during one passage through the kidney measures the renal plasma flow. The clearance of this substance will be independent of its concentration in the plasma providing this does not exceed the capacity of the tubules. When the plasma concentration exceeds the saturation level of the tubules, further excretion becomes referrable only to filtration, and the clearance is depressed.

In figure 6 the clearance of PAH, C_{PAH}, is plotted against the 50 minute plasma concentration. The data were obtained from the same 60 animals discussed above. It is evident that the clearance is independent of plasma level where this is not less than 4.5 mgm. per cent nor more than 7 mgm. per cent.

Above 7 mgm. per cent the clearance is progressively depressed while, on the other hand, abnormally high clearance values are obtained when the concentration falls below 4.5 mgm. per cent. This latter finding is interpreted as a confirmation of the caution stated previously, that a 50 minute plasma level below tubular saturation probably reflects a falling curve during the clearance period. Such end point values are lower than the true mean and hence yield falsely high clearance values.

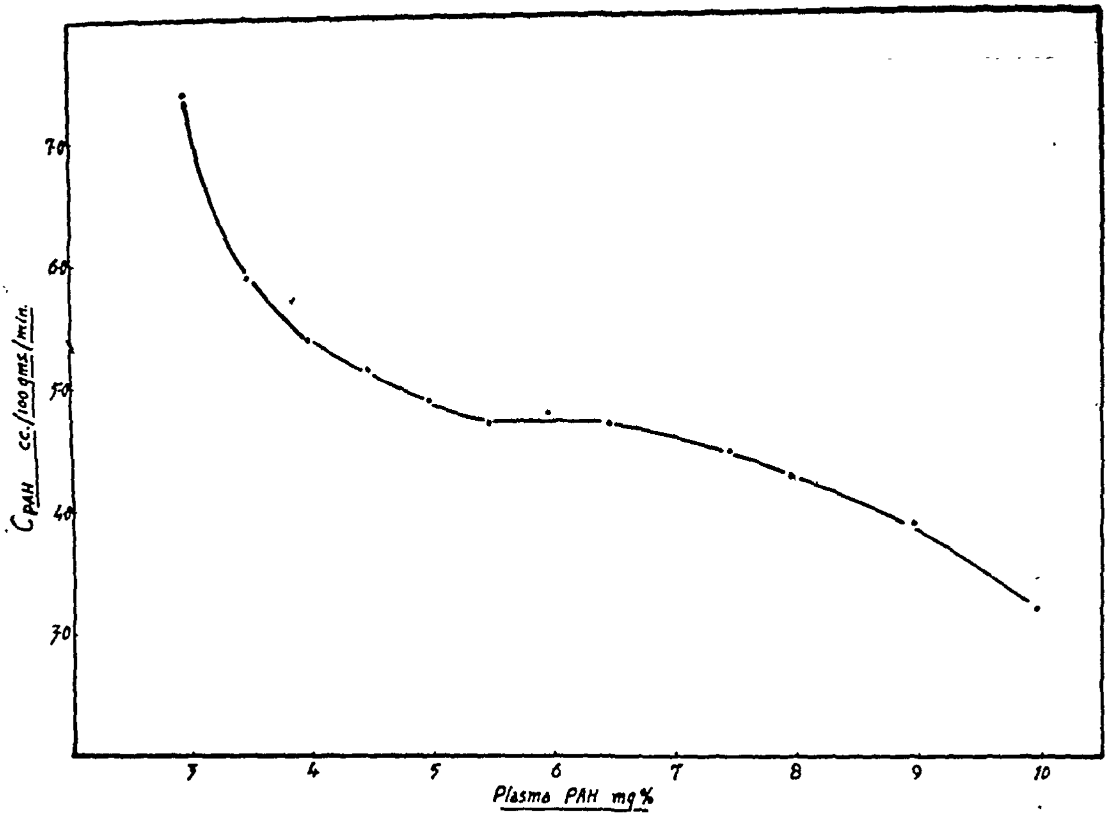

Fig. 6. The clearance of PAH at various plasma concentrations. Each point is the average of determinations in several animals weighing 120 to 160 grams.

c. *The measurement of tubular mass.* The tubular excretion, T, of a test substance is the minute urinary output of that substance less the amount excreted by filtration. In calculating T, since the water content of plasma, W, is relatively constant at about 0.9, it is taken as unity. Similarly, for those plasma concentrations under discussion a negligible error is introduced by considering the freely filterable fraction of PAH, F, to be constant at 0.8 (4).

Once the tubules are saturated, T becomes a constant, Tm, which reflects the amount of functional excretory tubular tissue. As shown in figure 7 the saturation level, where T becomes a constant, is again 5 to 7 mgm. per cent. Beyond 7 mgm. per cent a secondary rise in T is observed. This is expected, since no correction was made for the change in F as the plasma concentration

increased. Moreover, above tubular saturation the curve of plasma concentration for the period is humped and the 50 minute plasma level used in the calculation of T is lower than the actual mean plasma concentration for the period. It is important, therefore, in the study of clearance values in normal animals that the terminal plasma level of PAH lie within a restricted range, if values derived are to have absolute as well as relative meaning.

Procedure for the simultaneous determination of inulin and PAH clearances in large series. In designing a clearance procedure applicable to the study of large

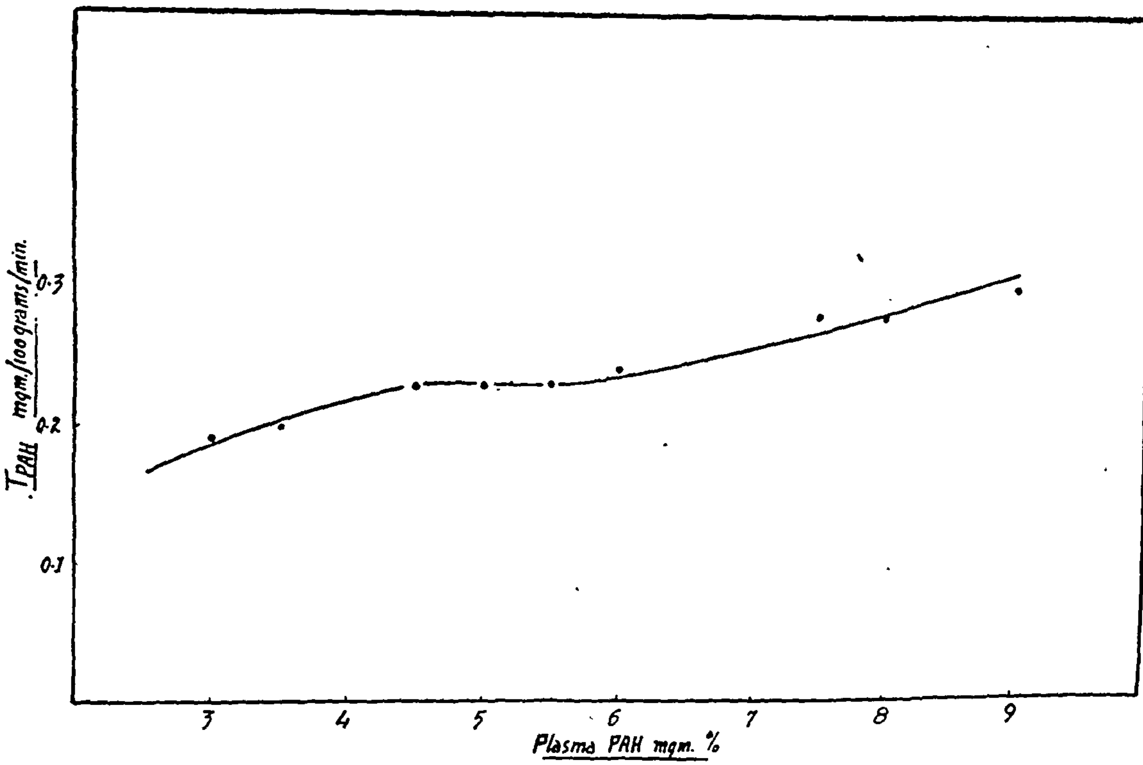

Fig. 7. Tubular excretion of PAH at various plasma concentrations. Each point is the average of determinations in several animals weighing 120 to 160 grams.

series of animals the method should be as simple as possible. To effect this the inulin and PAH analyses were modified so that they could be carried out simultaneously as far as possible. The animal and analytical procedures were as follows.

Animal methods. Four cubic centimeters of PAH solution (12.5 mgm./cc. in 2 per cent sodium sulfate) are injected subcutaneously in the lumbar region. The 50 mgm. dose will yield the correct plasma level in almost all animals of 160 to 210 grams. If the weight is 120 to 160, a 45 mgm. dose suffices, while for animals of 220 to 260 grams the dose may be raised to 55 mgm.

Immediately following this, 3 cc. of warm inulin solution (2 per cent inulin in saline) is injected intraperitoneally. Although the inulin solution in the earlier experiments was treated to remove pyrogen, we have latterly found this unnecessary. The collection period

is timed from the completion of the second injection. The rat is placed directly into a metabolism funnel. Fifty minutes after injection the rat is picked up over the funnel and the bladder drained by suprapubic pressure. Micturition is usually quite free and spontaneous, however. Immediately following urine collection 0.75 cc. of blood is obtained by heart puncture, using a 24 or 25 gauge needle. With practice, the blood is taken during the 51st minute. The animal is then returned to its cage unharmed and the blood centrifuged for 15 minutes at high speed.

The funnel is rinsed thoroughly and urine and funnel washings made up to a total volume of 100 cc., 0.2 cc. of which is used for the analytical procedure. If the diluted urine is heavily contaminated with feces it may be cleared by filtration; under ordinary circumstances, however, it remains reasonably clear. Attempts to filter the urine directly by devices within the funnel are not advisable since there may be the loss of a drop of urine containing a high concentration of the test substances. Urine made up as above yields a negligible blank value for both PAH and inulin despite feces contamination.

Chemical methods. 1. Add 0.2 cc. plasma to 6 cc. distilled water, rinsing the pipette thoroughly.

2. Add 2 cc. of 13 per cent trichloracetic acid. Shake and centrifuge at moderate speed for 5 minutes.

PAH

3a. To 3 cc. of supernatant add 0.3 cc. of 0.1 per cent sodium nitrite. Shake and allow to stand for 3 minutes.

4a. Add 0.3 cc. of 0.5 per cent ammonium sulphamate. Shake and allow to stand for 3 minutes.

5a. Add 1.5 cc. of 0.1 per cent N-(naphthyl) ethylene diamine dihydrochloride. The color develops rapidly. Allow to stand 10 minutes, then transfer to a micro colorimeter tube for reading in a photoelectric colorimeter using a 525 μ filter. The colormeter is set to zero with a colorless reagent blank.

Inulin

3b. Place 2 cc. of supernatant in a boiling tube.

4b. Add 4 cc. of Harrison's reagent

5b. Place the tube in boiling water for 30 minutes, cool at room temperature for 30 minutes, transfer to a micro colorimeter tube and read in photoelectric colorimeter with a 625 μ filter. The colorimeter is set to zero with a reagent blank.

Experiments with rat plasma show a negligible blank for PAH and a small relatively constant blank for inulin where the solutions are free of turbidity. At inulin plasma levels above 30 mgm. per cent the blank value may be ignored.

Urine diluted as stated in the section dealing with animal methods is handled in the same way as plasma, 0.2 cc. being further diluted in 6 cc. of water and exposed to the precipitating agent.

Using this technique, the animals are conveniently handled at the rate of 16 a day by one operator and one analyst.

In calculating the normal clearance values obtained where the terminal PAH level is between 5 and 7 mgm. per cent it was observed that renal function did not increase in direct proportion to body weight, so that expressing results in terms of 100 grams yielded lower values for larger animals, as seen in table 3. This difference disappeared if the results were corrected according to kidney weight using the formula of Braun Menendez (9). The formula, y (kidney weight) $= 2.06\ x$ (body weight) $+ 277$, is in reality only a special case of the general power formula $y = ax^b$, where a and b are constants; kidney weight is

actually a function of surface area. Meeh's application of the power formula to surface area in the rat, $y(\text{cm}^2) = 11.23 \text{ weight}^{2/3}$, was applied to the data and table 3 shows the identity of values obtained for the two series of animals when the data were recalculated for 100 cm².

TABLE 3

GROUP	NUMBER OF ANIMALS	WEIGHT	C_{IN} CC./100 G.	C_{PAH} CC./100 G.	Tm_{PAH} MGM./100 G.	C_{IN} CC./100 CM².	C_{PAH} CC./100 CM²	Tm_{PAH} MGM./100 CM²
		gms.						
1	8	142	0.84 ± 0.06	5.02 ± 0.36	0.23 ± 0.01	0.39 ± 0.02	2.32 ± 0.17	0.10 ± 0.01
2	8	177	0.69 ± 0.10	4.40 ± 0.34	0.19 ± 0.01	0.36 ± 0.04	2.31 ± 0.18	0.10 ± 0.01

TABLE 4

NO.	WT.	C_{IN} CC./100 CM²	C_{PAH} CC./100 CM²	Tm_{PAH} MGM./100 CM²	$\frac{C_{IN}}{C_{PAH}} \times 100$	$\frac{C_{PAH}}{Tm_{PAH}}$
					%	
1	155	0.36	2.16	0.11	16.7	19.1
2	154	0.38	2.20	0.10	17.5	21.2
3	146	0.39	2.40	0.10	16.6	22.4
4	153	0.36	2.59	0.10	13.8	25.6
5	147	0.40	2.45	0.11	16.5	22.0
6	148	0.42	2.33	0.12	18.0	19.2
7	140	0.42	2.07	0.10	20.4	19.7
8	140	0.41	2.41	0.10	17.1	24.1
9	175	0.29	2.13	0.09	13.6	22.5
10	168	0.39	2.32	0.10	17.0	22.3
11	186	0.36	2.23	0.10	16.4	22.0
12	188	0.35	2.18	0.08	16.0	23.0
13	175	0.27	2.14	0.10	13.1	21.7
14	168	0.36	2.32	0.09	17.0	25.2
Average		0.36	2.31	0.10	15.7	22.1
S.D.		±0.04	±0.18	±0.01	±2.3	±2.0

In table 4 normal clearance values obtained from 14 animals selected at random from our large series are shown. The results group closely as shown by the low standard deviation in each case.

Discussion. In the original attempt to apply the inulin and diodrast clearance methods to the rat the importance of a representative plasma sample was recognized. A clearance period of one hour was used and a mid point blood sample obtained. Since one hour had been allowed for equilibriation of the test substance before beginning the clearance period it was felt that the slope of fall of the plasma level during the second hour would be uniform and a mid point sample would accurately represent the mean. While consistent results were

obtained with the method it was admittedly laborious. Dicker and Heller (2) modified the technique to use a short clearance period of 10 to 30 minutes and introduced the device of a terminal blood sample as an estimate of the mean. It was not practical to test the validity of this end point determination since the diodrast analysis required sizable amounts of plasma and precluded the possibility of studying a plasma curve.

More recently Meyer Friedman (3) has studied the clearance of PAH in the rat and arrived at certain interesting conclusions. His method is open to considerable criticism, however, in the light of the present findings. For example, he studied the clearance of 100 mgm. of PAH in the hour following subcutaneous injection. This amount gives a markedly humped plasma curve and is well above the saturation limit. We cannot but feel that the ensuing end point plasma levels, as published, are essentially meaningless.

Dicker and Heller reported no variation in the inulin clearance with urine flow in the range studied. The present observations confirm these findings for low rates of urine formation (up to 0.75 cc./hr.). On the other hand, both Friedman (3) and Braun Menendez and Chiodi (10) have shown an increase in the inulin clearance with higher flows. Estimates derived from Friedman's data indicate that C_{PAH} is actually increased by the excessive hydration, and this increase in renal plasma flow would seem the obvious explanation for the increased glomerular filtration rate observed. It does not seem over-cautious to assume that urine flow rates exceeding 1 cc. per hour involve far reaching changes in renal hemodynamics which invalidate any attempt to interpret the clearance values obtained.

The clearance data of Braun Menendez and Chiodi are especially interesting. Their data are complicated by the presence of hydration since they were interested in this particular feature, but, in general, using a technique similar to that of Dicker and Heller they obtained values for C_{IN} and Tm_D of the same order as those here presented. Similarly, C_D was comparable to the normal value here given for C_{PAH} when urine flow was of similar magnitude and plasma diodrast did not exceed 3.5 mgm. per cent iodine.

In the present series the normal renal plasma flow, C_{PAH}, is higher than that previously reported and the C_{PAH}/Tm_{PAH} ratio higher than in any other species yet studied. It is not unexpected that this should be so when, for example, the normal pulse rate in the rat is approximately 300 per minute (11) when compared with 70 for man.

SUMMARY

1. Renal function has been investigated in the rat using inulin and sodium p-aminohippurate.

2. The calculation of results is most suitably based on surface area. The normal values obtained, expressed per 100 cm² of body surface are as follows: C_{IN} 0.36 $\pm$ 0.04 cc./min.; C_{PAH} 2.31 $\pm$ 0.18 cc./min.; Tm_{PAH} 0.10 $\pm$ 0.01 mgm./min.; F. F. 15.7 per cent $\pm$ 2.3; and C_{PAH}/Tm_{PAH} 22.1 $\pm$ 2.0.

3. Renal plasma flow per unit of tubular excretory tissue is greater than in other animals investigated and may be related to hemodynamic differences.

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RECOVERY OF A PRESSOR PRINCIPLE FROM THE BLOOD PLASMA OF CATS GIVEN KIDNEY EXTRACTS

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In a previous communication (1) a pressor principle was described which was found to appear in the blood plasma of cats that had died of several causes or that had been subjected to a period of hemorrhagic hypotension. The pressor principle was found in the plasmas of cats in which the kidneys remained intact but not in the plasmas of cats which had previously been nephrectomized. This pressor substance appeared to be characterized by its ability to cause a sustained elevation of blood pressure (BP) when injected intravenously into cats nephrectomized 1 to 2 days before, and contrariwise, the elevation in BP was slight and not sustained when the same material was injected into normal non-nephrectomized cats. These findings suggest that the kidneys are concerned in the production of this pressor principle and that they may also be the source of its inhibitor or antagonist.

It was also reported (1) that when kidney extract containing renin was injected into nephrectomized cats the resulting pressor response often did not subside completely, a sustained elevation of BP 10–15 mm. Hg above the base level being observed.

This observation suggested the possibility that the kidney extract might contain the sustained pressor principle in addition to renin, and that if larger quantities were administered over a longer period of time a substantial quantity of the pressor principle might be recovered from the cats' blood. Such a finding would offer further support for the renal origin of the principle.

METHODS. The kidney extracts were prepared from kidneys removed from normal cats under anesthesia. One hundred grams of whole kidneys were stirred in a Waring Blendor with 300 ml. of 2 per cent sodium chloride solution, after which glacial acetic acid was added to a concentration of 2 per cent. The insoluble material was removed by centrifugation and discarded. To the supernatant fluid ammonium sulfate solution was added to 0.6 saturation. The resulting precipitate was collected on Buchner funnels, suspended in water, and dialyzed 16 hours or until free of ammonium sulfate. The supernatant fluid from the material remaining in the sac was used as the kidney extract.

The extracts were injected intravenously into 3 series of anesthetized cats: 1, unoperated normal animals; 2, those bilaterally nephrectomized approximately one-half hour before, and 3, those nephrectomized 2 days before. The extracts (representing 13–34 grams of original kidney) were given in repeated doses over a period of 1 to 2 hours and, after varying amounts of time following the last injection, the cats were bled using heparin as the anticoagulant.

The plasmas obtained were assayed for their ability to cause a sustained pressor response. For this test, 2 ml. quantities were injected intravenously into pithed cats, the kidneys of which had been removed 2 days before. The technic for preparing the animals has been described elsewhere (1). With one exception each plasma was tested on two or more cat preparations and its activity evaluated by measuring the sustained elevation in BP in millimeters of mercury (see legend, fig. 1).

RESULTS. In figure 1 is presented a summary of the pressor assays of the cat plasmas. Those from the "2-day nephrectomized" cats were, in general, quite

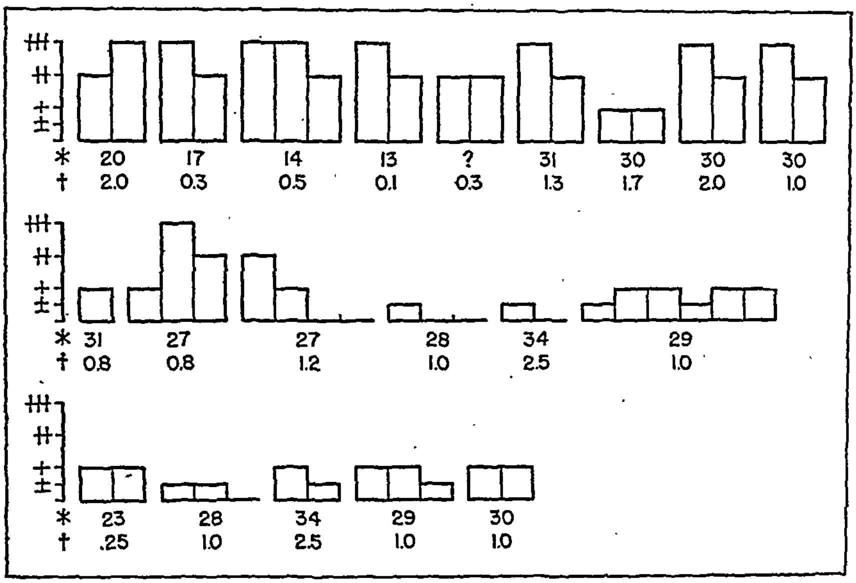

Fig. 1. Graphs showing sustained pressor activity of plasmas recovered from 3 groups of anesthetized cats given cat kidney extract intravenously; upper row, "2-day nephrectomized"; middle row, acutely nephrectomized; bottom row, non-nephrectomized. *—number of grams of original cat kidney from which the kidney extract injected was derived. †—number of hours elapsed after last kidney extract injection before cat was bled to obtain plasma. Each bar represents an individual test in a separate pithed cat. Ordinate scales indicate magnitude of sustained pressure elevation. ± 5–10 mm. Hg; + 10–25 mm. Hg; ++ 25–45 mm. Hg; +++ 45–65 mm. Hg; ++++ 65 mm. Hg or greater.

active and the sustained pressor responses compared in magnitude with those obtained with the injection of hemorrhagic shock plasmas (1). The plasmas from both the non-nephrectomized and the acutely nephrectomized cats usually showed distinctly less activity with only minimal or 1-plus sustained pressor responses. The plasmas from the acutely nephrectomized animals did not exhibit significantly greater sustained pressor activity than those from the non-nephrectomized cats.

Since large quantities of renin were present in the kidney extracts administered to the cats, the amounts of renin remaining in the plasmas were determined.

For the renin assay, 3 ml. of substrate solution[1] were incubated for 1 hour at 37°C. with 1 ml. of saline and 1 ml. of cat plasma made angiotonase-free by the method of Braun-Menéndez *et al.* (2). Dilute HCl was then added to pH 5.1–5.3 and the mixture heated to 100°C. for 10 minutes. The coagulated protein was removed by centrifugation and an aliquot of the clear supernatant fluid injected into pithed cats. Control mixtures were similarly made from the same materials, but were incubated separately. By this method of assay, only a very small amount of renin was found in any of the active cat plasmas and in several the presence of renin was barely detectable.

Evidence that the sustained pressor activity of the plasma was not due to the small quantity of renin present was obtained by injecting into a test cat an amount of diluted kidney extract which was shown by assay to have the same renin content as that of 2 ml. of the cat plasma. A transient, almost negligible elevation

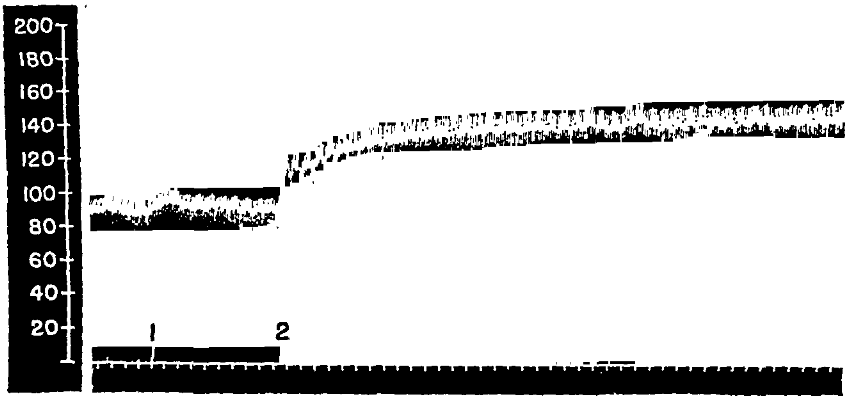

Fig. 2. Mean blood pressure tracing of pithed cat nephrectomized 2 days before. IV injection: 1, 0.002 ml. of cat kidney extract; 2, 2 ml. of plasma obtained from a "2-day nephrectomized" cat 2 hours after last injection of kidney extract, total quantity of which was derived from 30 grams of kidney tissue. The 0.002 ml. of kidney extract contained the same quantity of renin as the 2 ml. of cat plasma. Time marker—1 minute.

in BP resulted from the kidney extract injection, but the plasma produced a marked and sustained BP rise (see fig. 2).

Attempts were made to produce the sustained pressor principle *in vitro* by incubating for 3 hours, at 37°C., mixtures of small quantities of kidney extract and plasmas from cats nephrectomized 2 days before, with or without the addition of angiotonase prepared from hemolyzed red cells. Injection of the incubated mixtures into test cats caused transient elevations of BP which were no different from those resulting from the injection of the kidney extract alone.

Discussion. The present experiments offer further support for the belief that the kidneys are concerned in the production of the sustained pressor prin-

[1] The renin substrate prepared from hog serum was made angiotonase-free by adjusting to pH 3.5 with lactic acid and incubating 20 minutes at 37°C. The solution was then neutralized with NaOH.

ciple. Evidence now available indicates that it may be a new and separate entity, distinguishable by several means from other known pressor substances originating in the kidney. In favor of the hypothesis that the kidney extract contains the pressor principle in addition to renin are the observations that: 1, small but definitely sustained elevations of BP are observed when very small quantities of kidney extract are given intravenously to "2-day nephrectomized" cats; 2, large amounts of the pressor principle but only very small amounts of renin are recovered from the plasma of cats given large quantities of kidney extracts; and 3, incubation of kidney extract with blood plasma obtained from "2-day nephrectomized" cats does not cause the formation of detectable amounts of the sustained pressor principle. With intravenous administration, the animal may be able to destroy, fix in the tissues, or otherwise inactivate the renin, leaving unaltered the more durable pressor principle in the blood stream. However, the possibility has not been excluded that the enzyme, renin, may form another pressor agent which is much more stable *in vivo* than angiotonin, but, unlike angiotonin, is labile to heat *in vitro* and is nondialyzable (1).

The relatively large yield of active pressor material obtained from "2-day nephrectomized" cats and the recovery of only small amounts from the normal cats given kidney extract imply a possible inhibitory influence attributable to the presence of the kidneys in the latter animals. However, removal of the kidneys immediately prior to the injections of kidney extract did not significantly increase the amount of pressor material in the cat plasmas. The actual presence of the kidneys in the animal therefore cannot be regarded as the factor primarily responsible for the diminished yield of the active pressor agent.

SUMMARY

Kidney extracts prepared from normal cat kidneys were injected intravenously into three series of anesthetized cats: 1, unoperated normal animals; 2, those bilaterally nephrectomized one-half hour before, and 3, those nephrectomized 2 days before. Plasma from blood drawn varying periods of time following the injections was tested for the presence of the pressor principle capable of causing a sustained elevation of blood pressure in test cats which had been nephrectomized 2 days before.

Relatively large quantities of the pressor substance were found to be present in the blood plasmas from the "2-day nephrectomized" animals, while only small amounts were present in plasmas from the normal cats and from those which had been nephrectomized immediately before the kidney extract injections. The actual presence of kidneys in the animal does not appear to be the factor primarily responsible for the diminished quantities of sustained pressor material recovered in the latter plasmas.

The amount of renin remaining in the plasmas from the injected cats was very small and in several instances was barely detectable. Control injections of such minute amounts of renin into test cats caused only small, unsustained pressor responses.

The present experiments suggest that the sustained pressor principle may be a new and separate entity, physiologically distinct from renin, but extractable from kidney tissue along with renin.

We wish to acknowledge the technical assistance of Robert M. Sanders, William R. Cherry and Ora A. Harvey.

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THE CAROTID-MANDIBULAR REFLEX IN ACUTE RESPIRATORY FAILURE[1]

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Gasping in the isolated ischemic rat head has been used to determine the influence of drugs and of other factors on survival of this primitive respiratory mechanism during asphyxia (1–5). The advantages of this simple technique, which requires little time and less equipment for the study of various phases of respiratory center activity, are obvious. The purpose of these investigations is to examine the neurological basis of the mandibular respiratory movement, to which the meaning of the term "gasp" will be restricted, and to analyse the effects of oxygen lack and carbon dioxide excess.

METHODS AND RESULTS. *Discharge time.* Selle has shown that the survival of gasping is inversely proportional to age for animals, up to or slightly past the weaning period. A graph of this function for the animals in our colony was established (fig. 1) as a base of reference. The method and apparatus used for decapitation were similar to those described previously (4). Since there is no sex variation within any given age group, both male and female rats, of the Wistar strain, were used. They received no special treatment prior to decapitation. All measurements are given in terms of "discharge time," the interval between the first and last gasps. This measurement was used rather than survival time, measured from the time of decapitation, because it represents a unit of respiratory response, which indicates a certain minimum activity of the respiratory center. It is comparable in procedures which induce gasping after widely varied times, such as decapitation and nitrogen inhalation.

It has been shown that, in young animals particularly, two series of gasps, separated by a period of apnea, occur (1, 4). The use of substances preventing oxidative or anaerobic reactions has shown that the first series of gasps depends on aerobic mechanisms, the second on anaerobic ones.

Figure 1 represents a total of 220 animals evenly distributed throughout the age range. The curve showing discharge time also represents, of course, the end of the second series. The initial period of apnea following decapitation averages 4 seconds (3.2–5.0); the first series of gasps averages 3 seconds (0.5–6.7) and 4.5 gasps (1–8); and the period of apnea between the two gasp series averages 16 seconds (13.5–20.0). All these values are constant throughout the age range. In contrast, the second series of gasps decreases in duration and number, which vary together, by over 25-fold between one and five weeks of age; and thus controls variation in discharge time. A separate brain enzyme system promoting

[1] The present investigation was aided by a grant from the Dr. Wallace C. and Clara C. Abbott Memorial Fund of The University of Chicago.

anaerobic oxidations is probably involved in the second series. Its value to the relatively hypoxic fetal organism is obvious.

Reflex nature. In a preliminary report (6) it was shown that only when the carotid bodies remain connected to the brain does gasping occur. This was established for the decapitate rat and the dog with clamped trachea. To further

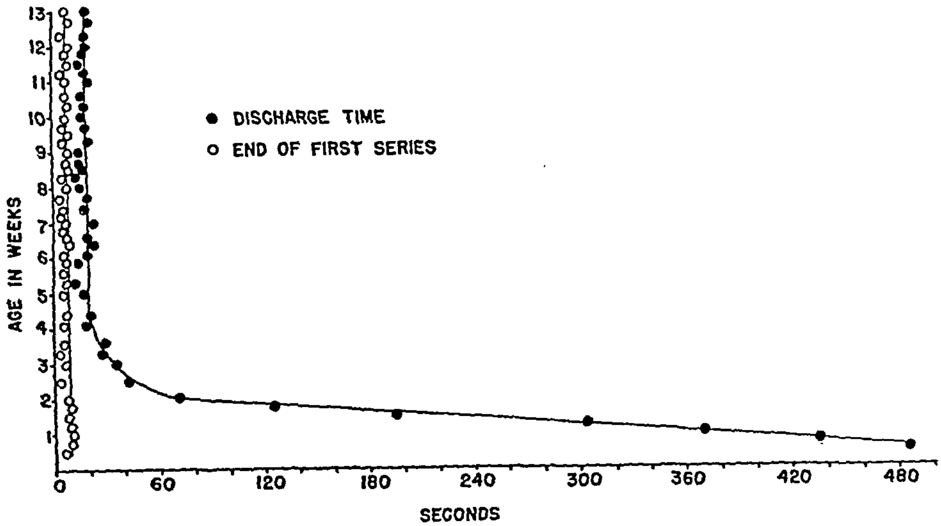

Fig. 1. Showing that variation of mandibular time with age in the untreated isolated ra head is entirely a function of the second anaerobic series of gasps. Each pair of points represents the mean value from five rats of the same age. Discharge time is measured from the first gasp; not from the point of decapitation.

TABLE 1

Showing the necessity of the carotid reflex mechanism in gasping produced by tracheal occlusion or cyanide poisoning

Rats were four weeks old. Bracketed figures give range.

	Gasping produced by tracheal occlusion			Gasping produced by cyanide poisoning		
	Number of rats	Average discharge time	Average number of gasps	Number of rats	Average discharge time	Average number of gasps
		sec.			*sec.*	
Carotid mechanism intact	10	138 (126–144)	20 (17–24)	10	851 (709–883)	153 (141–163)
Carotid mechanism denervated	10	0	0	10	0	0

substantiate the reflex nature of gasping, when produced by procedures other than decapitation, tracheal occlusion was performed on rats with denervated carotid bodies and the discharge times recorded. Four-week old rats were operated under chloralose, which has been shown to potentiate carotid reflexes and increase discharge time (6). In ten rats, denervation was completed; in ten similar controls a dummy operation was performed. As shown in table 1, only

the control group showed gasping. The denervated animals developed hyperpneic respiratory movements after occlusion of the trachea, but at no time did mandibular movements accompany these efforts.

In a similar series, gasping due to cyanide poisoning was also shown to be entirely a carotid reflex response. All animals given potassium cyanide (10 mgm. per kilo i.p.) died, but only those with innervated carotid bodies gasped (table 1).

The respiratory center. Having established the character of the carotid-mandibular respiratory reflex, the question arises as to which link in this reflex arc is the first to fail during anoxia and asphyxia. Since the carotid body chemoreceptors continue to discharge maximally for 20 to 30 minutes after death (7), one may safely assume that gasping does not cease through failure of these elements. That the efferent limb of the arc is similarly still functional when gasping ceases, was shown by testing the linguo-mandibular reflex. This also involves the motor muscles and peripheral connections of nerves V and VII, and could be elicited for a short time after gasping had ceased. Possible current leakage from the stimuli applied to the tongue was excluded by careful insulation. The respiratory center, then, must be the weakest link in the reflex chain, and discharge time is a measure of survival of that aspect of the respiratory center concerned with the gasping reflex.

Control of gasping. In the decapitate preparation, the tension of oxygen in the respiratory center and carotid bodies begins to fall, and of carbon dioxide to rise, at once. The rôles of hypoxia and hypercapnia in gasping and the locus of action were determined as follows.

A two-liter glass chamber was filled with a desired N_2-CO_2 mixture by water displacement, an 11-day old-rat introduced, and the system continuously flushed with the water-saturated gas mixture at 800 ml. per minute. Twenty animals were run at each of eight atmospheres. After placing an animal in nitrogen, a period of violent struggling began within twenty seconds and lasted for about fifteen. Near the end of this time, an intense apneic gasping began to accompany each inspiratory effort. The mandibular movements then gradually became less marked until they were no longer visible (discharge time endpoint), although contraction of the diaphragm and intercostal muscles continued for a short while. The average discharge times are shown in figure 3. In 100 per cent nitrogen this is 142 seconds, 58 seconds less than that after decapitation. (Controls of same strain, age, diet, season.) With increasing CO_2, to 35 per cent, anoxic discharge time increases, to a maximum of 320 seconds, and then declines again. Discharge time varies directly with the total number of gasps, the intervals between gasps being essentially constant.

It is concluded that anoxia alone can promote respiratory activity at the gasping level, but that maintenance of gasping is enhanced by hypercapnia, as in true asphyxia.

The gasping pattern elicited by exposure to anoxic and hypercapnic atmospheres indicates a continuous and constant level of discharge, with no grouping into the two separate series of the young decapitate preparation.

Nevertheless, iodoacetate (1.0 mgm. i.p., 15 min. before anoxia, 45 rats) cut the average discharge time to 20 to 30 seconds, regardless of carbon dioxide concentration. Since iodoacetate eliminates the second series of gasps in the decapitate preparation by interference primarily with the anaerobic oxidative mechanisms (4), it would seem that in the simple anoxic preparation, also, aerobic processes support the initial gasps, and anaerobic ones the later gasps. And, as in the variation of discharge time with age, the differences in discharge time with changing carbon dioxide tension reflect only the duration of the anaerobic series of gasps.

The effect of hypercapnia without anoxia was tested by placing similar rats in atmospheres of carbon dioxide and oxygen. With increasing carbon dioxide

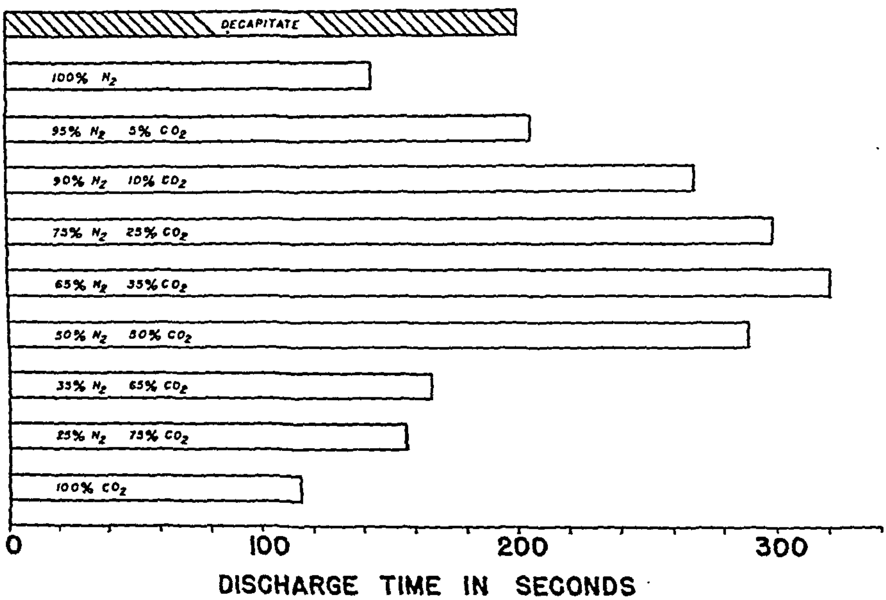

Fig. 2. Showing the effect of various carbon dioxide concentrations on discharge time of untreated 11-day old rats placed in O_2-free atmospheres. Each bar represents the mean value from 20 animals placed in the indicated N_2-CO_2 mixture. Lowest t value is 30. The shaded bar indicates discharge time for the comparable isolated head preparation.

concentration, up to 35 per cent, no gasps were evoked, even on prolonged exposure. Higher concentrations of carbon dioxide produced erratic gasping patterns and death. Since concentrations of this magnitude markedly decrease the oxygenation of the blood and interfere with tissue oxidations, a factor of hypoxia cannot be eliminated. It is obvious that hypercapnia alone has little ability to produce gasping. Anoxia supplies the main drive and CO_2 concentration modulates it.

To determine the site of action on the gasping reflex, a preparation was devised whereby the chemical environment of the carotid chemoreceptors and that of the respiratory center could be varied independently. This involved perfusing the carotid bodies, isolated from the main vascular system, with the circulation from another animal, similar to the method of Heymans, et al. (8). Two

male rats were anesthetized with chloralose. Both carotid forks of one were isolated and perfused by blood from and returning to the carotid arteries of the second (fig. 3). Since the respiratory center is chiefly supplied by the vertebral and spinal arteries, ligation of the common carotid arteries of rat 1 caused little impairment of medullary circulation. Heparin (0.02 gram per kilo i.v.) was administered to rat 2 at the end of the operation.

Rat 2 functions as a perfusion pump and also, since its own carotid bodies are intact and receiving their usual blood supply, as an indicator. When it gasps, the blood perfusing the carotid bodies of rat 1, as well as its own, is obviously

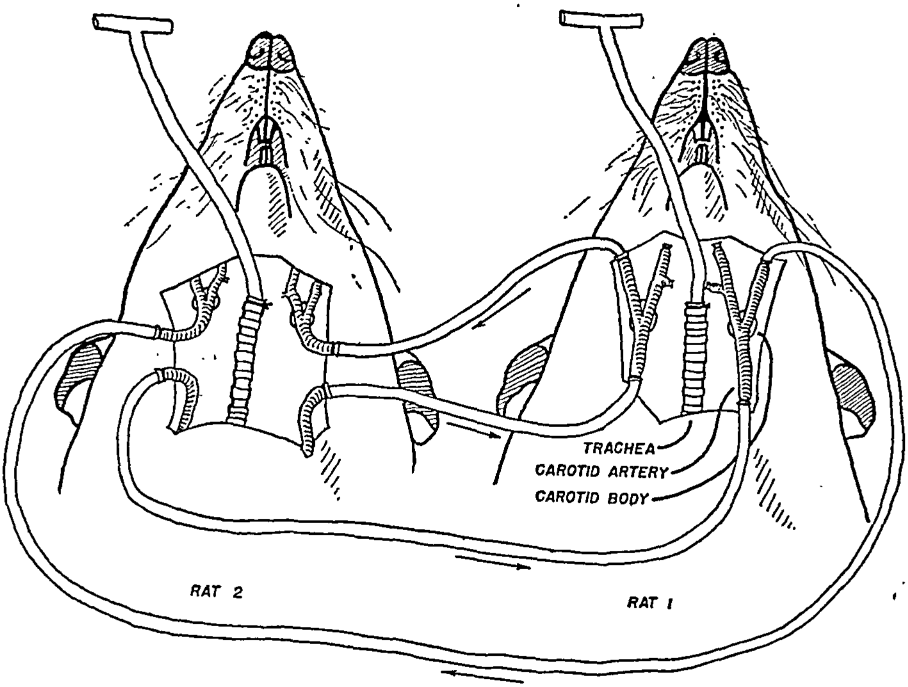

Fig. 3. Diagram of the method used to perfuse the carotid body of one rat (white male 200 grams) with blood from a similar animal. The common and internal carotid arteries were cannulated with polished hypodermic needles and rubber tubing was used for connections. Rat 2 is heparinized to prevent coagulation. The T-tube tracheal cannulae lead to valves by which different atmospheres can be administered.

capable of initiating the gasping reflex. A desired gas mixture was administered to each animal from a rubber bag attached to a T-tube in the trachea with appropriate one-way valves.

The results of such experiments are summarized in table 2. It is clear that CO_2 stimulation of the carotid body cannot initiate gasping unless the neural centers are anoxic. Conversely, with an anoxic center, the full potentiation by CO_2 can be obtained when this acts only on the carotid system. Thus, in the simple inhalation experiments, 35 per cent CO_2 increased discharge time 129 per cent; and, in the perfusion experiments, 35 per cent CO_2, acting only on the carotid body, increased it 141 per cent.

Discussion. Although gasping has little or no function in normal respiration of the mammal, it nevertheless provides an index of neurone activity. As such, and because of its unique ability to distinguish functionally between glycolytic and aerobic oxidations, the technique described may prove useful in investigating the metabolism of the respiratory center. Although the animal is *in extremis* when gasping is observed, the conditions are probably still more physiologic than with in vitro methods.

An analysis of gasping is of value not only in relation to the use of this phenomenon as an indicator of metabolic activity, but also in relation to respiratory control. Respiratory movements of fish consist of little more than gasps, indicating the primitiveness of this response. Lumsden has described (9)

TABLE 2

Showing the effect of perfusing the carotid bodies of rat 1 with blood from rat 2 when each animal is inhaling a different atmosphere

Gasping of rat 2, when it inhaled N_2 or N_2-CO_2, indicates that the carotid bodies of rat 1 were sufficiently stimulated to evoke gasping. N_2 = 100% N_2; N_2-CO_2 = 65% N_2-35% CO_2. N_2-CO_2 is referred to as asphyxic. Four experiments were done at each inhalation pattern. Bracketed figures give the range of the four results.

INHALED GAS		CONDITION OF RAT 1		RESPONSE OF RAT 1	
Rat 1	Rat 2	Carotid body	Respiratory center	Number of gasps	Discharge time
					sec.
Air	N_2	Anoxic	Normal	0	
Air	N_2-CO_2	Asphyxic	Normal	0	
N_2	Air	Normal	Anoxic	0	
N_2-CO_2	Air	Normal	Asphyxic	0	
N_2	N_2	Anoxic	Anoxic	5 (4–7)	16.5 (14–19)
N_2-CO_2	N_2	Anoxic	Asphyxic	6 (5–7)	15.0 (12–18)
N_2	N_2-CO_2	Asphyxic	Anoxic	12 (10–14)	40 (35–42)

a primitive gasping center located in the medulla and unresponsive to changes in carbon dioxide tension. It is possible that the carotid-mandibular reflex measures activity of this center, released as more recent centers succumb to anoxia in a reverse phylogenetic order (10). The alternative hypothesis that anoxia stimulates the central mechanism would seem to be contraindicated by the body of evidence against direct anoxic excitation of the respiratory center (11).

Two factors are necessary for the appearance of the carotid-mandibular reflex; anoxia of the respiratory centers, and stimulation of the carotid chemoreceptors. Potentiation of the response by carbon dioxide acting on the carotid end organs is probably due merely to stronger afferent bombardment of the medullary centers.

Preliminary work indicates that the variation of response time with age is a function of the central neurones and not of the carotid body.

SUMMARY

Gasping of the severed rat head has been used by several workers as an index of activity of medullary respiratory centers. Present experiments establish the reflex character of gasping in several types of anoxia and locate the actions of anoxia and hypercapnia in the reflex arc.

After decapitation, cyanide administration, or inhalation of oxygen-free gas mixtures, gasping occurs when the carotid bodies remain functionally connected with the medulla but is entirely absent when they are disconnected. Gasping is terminated by central failure, for the carotid receptors discharge long after gasping ends, and the lingual-maxillary reflex (engaging the same efferent arc) also outlasts it.

Discharge time (from first to last gasps) decreases with age. For 11-day rats inhaling pure nitrogen, it averages 142 seconds, as compared with 200 after decapitation. Addition of CO_2 to the N_2 (but not to O_2) increases discharge time, to a maximum 320 seconds at 35 per cent CO_2. That the prolongation depends on anaerobic processes is shown by the ability of iodoacetate to cut discharge time to 25 seconds, whatever the CO_2 concentration.

By "perfusing" the carotid bodies of one rat with the blood of a second and varying independently the gas inhaled by each, the medullary centers and the carotid bodies of an animal were exposed to different conditions. Anoxia both of the brain and the carotid body must be present for gasping to occur. The potentiating action of CO_2 is exclusively on the carotid body.

These findings, while establishing important peripheral factors in the gasp response, do not vitiate its use in investigating the action of central neurones.

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LOCAL SWEAT GLAND ACTIVITY DUE TO DIRECT EFFECTS OF RADIANT HEAT

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During a series of experiments designed to study the reflex sweating patterns induced by application of intense radiant heat to a relatively small area of skin, marked differences were observed in the size of sweat spots in the areas of highest and lowest temperatures (fig. 1). Sweat spots in the heated areas were distinctly larger and darker than those in adjacent, peripheral areas, indicating much greater sweat secretion by individual glands in the areas of high temperature. The smaller, lighter spots in the periphery were quite comparable in appearance to those elicited during normal reflex sweating. Sweat spots comparable in size to those in the area of high temperature are seldom observed in normal sweating except in specific areas of the body (axilla, etc.), or under conditions of maximal stimulation (very hot tub-bath or stimulation by cholinergic drugs, Randall, 1946).

Such marked differences suggested the possibility of differential responses of individual sweat glands to direct heating and to stimulation by reflex paths.

In order to investigate further this profuse sweating response of individual sweat glands, an apparatus was constructed to heat a small localized area without directly heating surrounding areas. The apparatus consists essentially of a loop of high resistance chromel wire through which a low voltage, alternating current could be passed. This unit is housed in a tube of about 1 cm. diameter which in turn is covered by several thicknesses of asbestos insulation. When this apparatus is brought into position over the test area, a region about 10 to 20 mm. in diameter is warmed to a high temperature while surrounding areas remain relatively normal. A temperature difference of 10 to 15 degrees C. on the skin surface may thus exist within a distance of 5 to 15 mm. This unit is in series with an adjustable transformer so that the voltage could be regulated and the amount of heat directed onto a small, sharply localized area of skin easily controlled. Individual sweat gland responses were recorded on paper according to a method previously described (Randall, 1946). Briefly, the method involves the starch iodine reaction at each sweat pore when a small droplet of sweat in the pore places iodine (painted on the skin) and starch (in a sheet of bond paper) in solution, resulting in a definite bluish-black spot on the paper. The duration of exposure of the paper over the skin was 20 seconds and exposures were made at 30 second or 60 second intervals.

Temperatures at the skin surface were recorded from copper-constantan junctions in holders especially constructed to permit normal evaporation and radiation heat loss from the skin even though the thermocouples were held in

firm contact with the skin. The holders were constructed out of polystrene bars (5 cm. x 1 cm. x 0.5 cm.), one end of which was forked. The unshielded thermocouple junction was stretched across this fork and held in firm contact with the skin surface without obstruction of heat loss, by taping the body of the holder to the skin. The points of the fork across which the junction was fixed extended a few millimeters below the body of the holder to further insure firm contact with the skin.

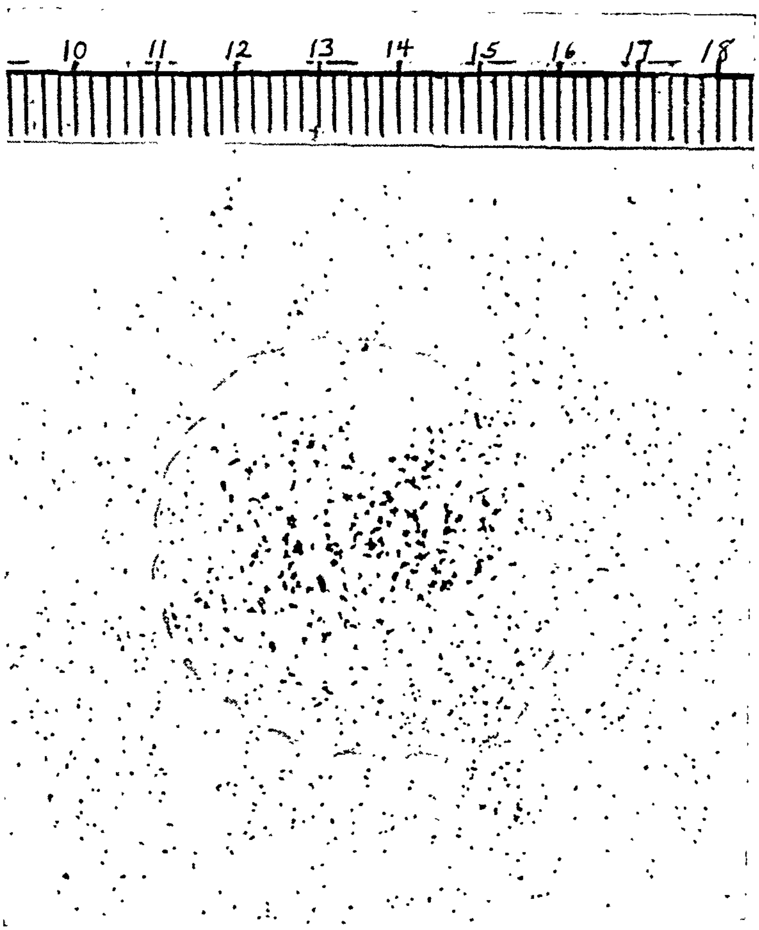

Fig. 1. Contrasting sweat responses in the center and around the periphery of a locally heated area. Broken line encircles approximate region of high temperature with highest temperatures attained in area of large spots. Calibration numbers in centimeters. Enlarged photograph of original record retouched with india ink for purposes of reproduction.

In many experiments in which considerable sweating was observed during the control period an initial inhibition of sweating was noted immediately after turning on the heat. This was not always noted however and was not noticeable, of course, when the heat was turned on during a low point in a cycle of sweating or when no sweating was evident during the control period. Ordinarily a short delay of 1 to 2 minutes (range of 1 to 8 min.) preceded active secretion of sweat glands following application of heat. During this period of delay the tempera-

ture on the skin surface rose quickly to temperatures considerably in excess of those normally occurring even in hot environments (range from 39 to 45°C). Since the couples were necessarily placed on the surface, however, the high temperature was attained here somewhat before the gland cells themselves were heated at some distance below the skin surface. This factor alone may explain in large part the delay in sweating responses behind the rise in surface temperature.

Following this delay a few very large, dark sweat spots appear in the area of highest temperature with little or no sweating on adjacent areas. Sometimes smaller sweat spots may be noted briefly on adjacent areas (fig. 1) but as long as the temperature of these areas remains low, and the local heating is not excessively painful, sweating does not persist except for the normal cyclic periods of activity. Sweating increases in intensity in the locally heated area and is marked by increasing numbers of large droplets (which appear on the record as large, dark spots, indicating excessive output by the individual glands). Cyclic activity in this area is depressed or obscured by the profuse sweating and many glands appear to be continuously active for the duration of the exposure to heat. If excessive heating is prolonged, 70 to 80 per cent of functional sweat glands in the area may be stimulated, but it was usually observed that the primary response to direct local heating was an increased output of individual glands rather than stimulation of a maximum number of glands.

When the heat was turned off both the size and number of sweat spots immediately decreased as local sweating declined more or less progressively with the fall in surface temperature. Sweating then disappeared when the skin temperature was still a few degrees above the starting normal. It may be suggested therefore that a critical temperature exists above which direct, locally induced sweating occurs and below which it does not occur. This critical temperature is several degrees above that necessary to induce sweating by reflex, when heat is applied to a larger surface area.

Figure 2 demonstrates this local sweating response to concentrated heat on the forearm. Note the relationship between sweating and local surface temperature. The fact that sweating is present at lower temperatures as the area cools compared with the period of heating is probably related to the insulation and heat storage capacities of the skin. In one experiment the subject complained of very severe pain during the heating period and sweating appeared diffusely spread over the heated and adjacent non-heated areas alike, simultaneously with the development of the severe pain. The rapidity of onset of this sweating together with the smaller and more discrete character of the sweat spots identified this response as purely reflex in nature, and contrasted with the delayed appearance of large sweat spots confined to the heated area.

In a few experiments it was noticed that after prolonged exposures to such local heat (at temperatures well above 40°C) sweating continued for as long as several hours in the central area of highest temperature. Such sweating continued in spite of the fact that skin temperatures returned to normal. In some experiments, the temperatures of these local areas were reduced several degrees

below the starting normal temperatures by application of ice-filled containers, without abolition of the persistent local sweating. In all such experiments, damage to the skin was evidenced later by the appearance of local edema and blister formation. In fact, after some experience the experimenter could predict

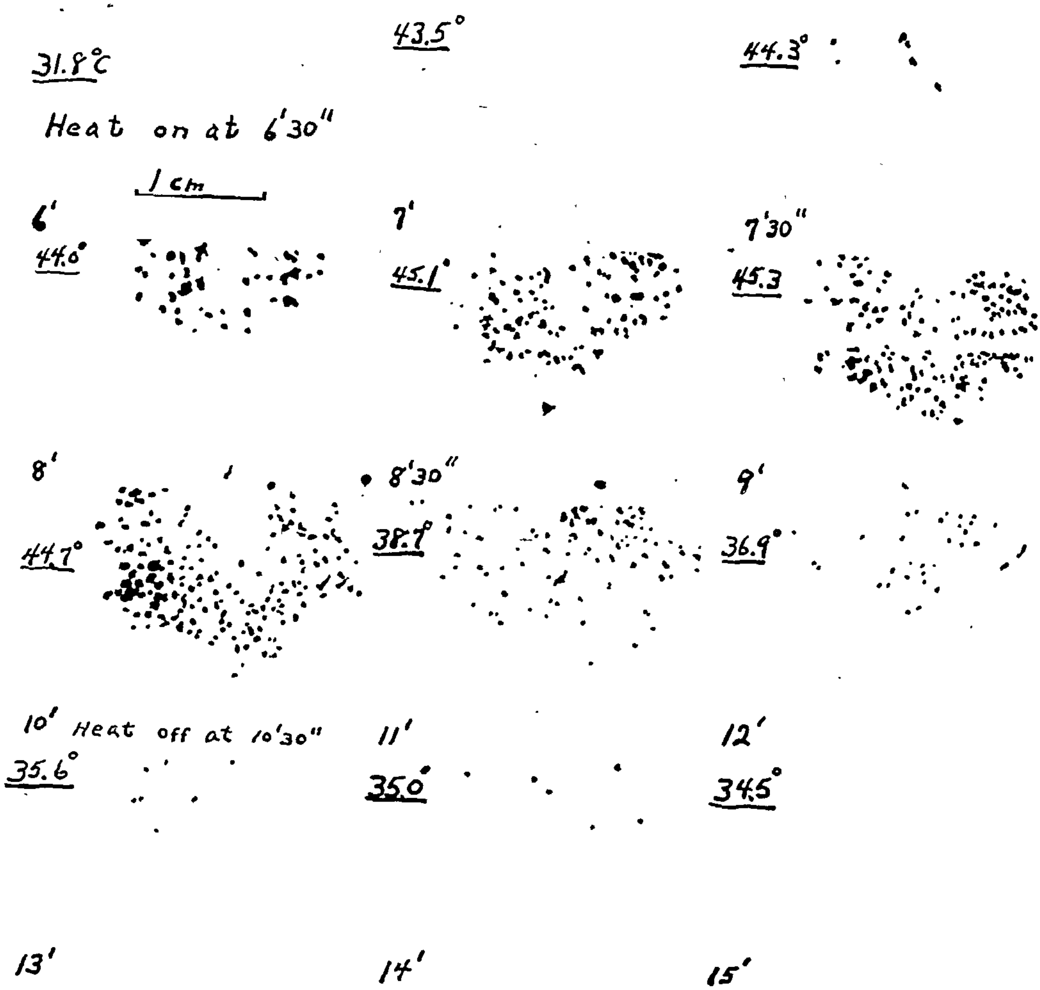

Fig. 2. Experiments demonstrating sharply localized response to local radiant heat on forearm. Records of 20 second duration taken at 30 second intervals. No sweating was present during first five minutes of control period, and successive records starting at sixth minute show development and regression of individual gland responses (individual sweat spots) during and following heat application. Enlarged photograph of original records retouched for purposes of reproduction. Skin surface temperatures of heated area shown in upper left corner of each record.

skin damage by excessive heat, even before blister formation was evident, by the persistence of local sweating.

Kuno (1934) reported that the irritability of the "sweat mechanism" including the centers in the central nervous system varies seasonally, probably involving processes of acclimatization. To our knowledge, however, there is no

evidence that such changes in critical temperatures of individual glands to direct· effects of heat occur. Table 1 summarizes the protocols of a number of experiments in which the skin temperature was rapidly elevated until local sweating was established. Since the temperatures were recorded from the skin surface rather than at the precise depth of the sweat glands, it is not advisable to consider the values as true threshold temperatures, and as indicated above, threshold values appear to lie at somewhat lower levels. The data do indicate however that such threshold levels of stimulation do exist. This is demonstrated clearly in subject Smi (2/7/47) where in successive experiments sweating failed to appear when the surface temperature was maintained at 41.15 to 41.85°C for

TABLE 1

SUBJECT	DATE	STARTING SKIN TEMPERATURE	TIME TO INDUCE FIRST LOCAL SWEATING	SKIN TEMPERATURE AT FIRST LOCAL SWEATING	
		°C			
Slo	5/3/46	34.25	1 min. 40 sec.	42.6—Room temperature 23–30° in	
Slo	5/6/46	33.10	1 min.	42.2 all summer experiments	
Slo	5/7/46	33.30	2 min.	41.0	
Ra	5/4/46	33.15	1 min. 30 sec.	43.2	
Ra	5/3/46	32.9	2 min.	42.6	
Ra	5/18/46	32.2	3 min. 30 sec.	39.6	
Ra	5/22/46	32.0	1 min. 30 sec.	45.5	
Ra	5/20/46	31.5	1 min. 30 sec.	43.7	
Ra	5/20/46	32.05	2 min.	38.4	
Ra	6/22/46	34.1	2 min. 30 sec.	45.35	
Ra	6/6/46	33.8	6 min. 30 sec.	42.9—Temperature raised slowly	
Hal	5/23/46	33.9	8 min. 30 sec.	45.5—Temperature raised slowly	
Ri	5/20/46	31.2	2 min.	43.8 Response delayed several	
Ri	5/20/46	32.7	2 min. 30 sec.	44.7 minutes after maximum	
Ri	5/20/46	33.4	2 min.	44.7 temperature had been attained	
Smi	2/7/47	30.2	No sweating	41.85 (maximum)	16.0—Room
Smi	2/7/47	31.2	2 min. 40 sec.	42.7	17.0 Temp.
Ra	2/7/47	30.0	1 min. 40 sec.	44.2	17.0
Ra	2/7/47	30.1	1 min. 30 sec.	41.4	16.0
Ri	2/25/47	31.7	1 min. 30 sec.	44.8	25.5
Dee	2/24/47	32.0	1 min.	44.3	21.0

6 minutes, but sweating did occur in a subsequent experiment as soon as the surface temperature reached 42.7°C.

Considerable variation in temperatures at which sweating may first occur, even in the same individual at different times, is indicated. These variations possibly may be referable to differences in temperature at the precise level of the contractile elements in the sweat glands.

Thus it is not possible to say that small changes in the threshold temperature at which sweat glands respond to direct stimulation do not exist in the general acclimatization of the sweating mechanism from summer to winter, but it appears from table 1 that if such changes do occur they are relatively small. It is

likewise impossible to explain relatively large differences in so-called thresholds (such as are apparent in subject Ra) at different times. It is evident however that the range of such variations are similar in the summer and winter experiments. If temperatures are raised more slowly sweating may occur at a somewhat lower temperature, but this is probably directly related to the temperature gradients induced and even in these experiments a threshold temperature must be reached before sweating occurs.

Evidence thus far presented indicates that the sweat glands will respond to directly applied heat, but the possibility of a sharply localized reflex arc is not completely ruled out. From the diffuse type of response observed in experimentally demonstrated reflex sweating (Randall, 1947) it is difficult to reconcile such sharply localized sweating (with complete absence of sweating in adjacent areas in some experiments) to anything but a direct response of the glands.

To check this, normal sweating was abolished in a small area (about 15 mm. diameter) by iontophoresis of atropine (pad soaked in 10 cc. of solution containing 1 mgm atropine, 2 ma for 2 to 5 min.) while sweating continued in all adjacent areas. Application of heat to the opposite arm caused excessive sweating on the normal, adjacent areas but no sweating in the atropinized area. Heat was then applied directly to the atropinized area. Although in no instance did sweating appear as pronounced in the atropinized area as in the adjacent tissue, a large number of glands were activated at about the same surface temperature as shown to be required to produce local sweating. Thus although reflex impulses were blocked entirely by atropine, some sweat glands in the area responded to direct application of heat. Similar results were observed when normal reflex sweating was abolished by intradermal injection of 2 per cent procaine, that is application of intense radiant heat to the wheal resulted in sweat responses.

Finally, the local sweating response to heat may remind one of the local response to faradic stimulation reported by Wilkins, Newman and Doupe (1938) and by Bickford (1938). These workers suggest that the local response to faradic stimulation represents an axon reflex mediated through postganglionic sympathetic fibers. It seems doubtful, however, that local heating as employed in the present experiments could act as a direct stimulant comparable to faradic stimulation. Further, the local sweating response to intense heat was not abolished (although it was decreased) by atropine and procaine. Such observations made on human skin agree with those of Saito (quoted by Kuno, 1934) who obtained sweating by warming the pads of the cat's foot even though the innervation had been sectioned three to six weeks before.

These observations would indicate therefore, that the profuse response of sweat glands to local radiant heat of high intensity is a direct one. The threshold temperatures at which this direct stimulation occurs is considerably higher however than those encountered under normal circumstances.

SUMMARY

Using a method designed to investigate activity of individual sweat glands, the sweating responses to locally applied radiant heat were studied. When

the temperature at the skin surface was rapidly elevated to high levels (39 to 45°C), large dark sweat spots appeared in the heated area, indicating excessive sweat responses by the individual glands. These spots contrasted sharply in size with those induced by normal reflex stimulation. A definite threshold temperature appears to exist, above which a direct response to local heat may occur and below which it does not occur. This threshold appears to lie within a rather wide range of temperatures and the range of variation does not appear significantly different in summer and winter. Pharmacological evidence is presented to suggest that the profuse sweating response of individual glands to extreme temperatures is a direct one in contrast to the usual diffuse reflex response.

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ALTERATIONS IN UROGASTRONE EXCRETION PRODUCED BY EXTIRPATION OF VARIOUS ENDOCRINE GLANDS[1]

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In 1939 it was reported that human urine contains a substance which, on intravenous injection in dogs, inhibits gastric secretion (1, 2, 3). At first this gastric secretory depressant named urogastrone was believed to represent renally excreted enterogastrone, an inhibitor of gastric secretion formed by the mucosa of the small intestine (4). However, it was shown that the removal of either the stomach or the duodenum did not eliminate the active substance in the urine (5) and even completely enterectomized dogs still produced urogastrone, although the output was less than normal (6). Later reports pointed out some chemical and physiological differences between urogastrone and enterogastrone (7). Nevertheless, the possibility still exists that part of the gastric secretory depressing activity of urine may be due to a chemically altered excretion product of enterogastrone.

Urogastrone was also found to differ in many respects from the anterior pituitary-like hormone (8) as well as from estrogen (9), pituitrin (10), kallikrein (9) and anthelone (8, 11) which likewise may be extracted from urine.

Although demonstrated first in human female urine (8), urogastrone may also be obtained from the male (2). Dogs' urine extracts exert a similar influence.

The problem that presented itself for investigation was the possible correlation between the content of urogastrone in the urine and the function of some of the endocrine glands (8, 11, 20).

METHOD OF STUDY. Extracts have been prepared from the urine of dogs from which various endocrine glands have been extirpated as well as from urine of normal animals.

Twenty-six female dogs, divided into the following six series, were used, and between 60 and 150 liters of urine were obtained from each series: 12 normal (urine collected separately from 2 equal groups), 6 oöphorectomized, 6 thyroidec-

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tomized plus oöphorectomized, and 2 hypophysectomized (urine collected separately from each dog).

Oöphorectomy and thyroidectomy were done in routine fashion under nembutal anesthesia. Complete thyroidectomy was accomplished with care to preserve the blood supply of the parathyroids.

The pituitary gland was removed by the transbuccal method (12, 13). A small opening was made with a dental drill in the avascular area of the roof of the mouth directly underneath the sella turcica.

The two hypophysectomized dogs each developed polyuria which lasted approximately three weeks. Collections of urine were started one week after removal of the pituitary gland and were continued for about four months. Therefore, the total amount of urine collected from the hypophysectomized dogs did not exceed appreciably the quantity obtained from two similar normal dogs. Autopsies of these 2 dogs, performed approximately one year after the hypophysectomies, revealed that no portions of the pituitary were grossly seen. Serial sections of 7 microns were prepared from the decalcified base of the skull. On the right side there were no pituitary remnants. On the left side some degenerated pituitary cells, showing karyolysis, and eosinophilic cells were found lining the base of the pituitary fossa. Also a large number of red blood cells and old blood pigment were seen. These remnants according to Dr. Gabriel Steiner, Neuropathologist at Wayne University, were not important as to the functional activity of the pituitary gland. The hypothalamus of each brain was also cut in serial sections and microscopically did not show any lesions.

Urogastrone was prepared by the procedure of Gray, Wieczorowski, Wells and Harris (9), a modification of the Katzman-Doisy method (14) of preparing the anterior pituitary-like hormone. The crude extract amounting to 8–12 grams from each 100 liters of urine, was purified giving a yield of 130–180 mgm. Doses of 1, 2 and 3 mgm. of the purified extract were administered intravenously in 1 per cent saline solution. In a few experiments larger amounts such as 5–10 mgm. were employed. Gray et al. (9) previously standardized the urogastrone dose as that quantity producing 50 per cent inhibition of the secretion of gastric juice.

The 6 preparations of urogastrone, one from each of the previously mentioned series of dogs, (see second paragraph this section) were each tested for their effect on gastric secretion stimulated by histamine in both Heidenhain pouch and gastric fistula dogs. Four fistula and five pouch dogs were employed. With fistula dogs one could deal with much larger amounts of gastric juice excluding those experiments in which the secretion was contaminated by bile or saliva. One cubic centimeter of 1–1000 solution of histamine diphosphate[2] was injected subcutaneously in each test. The dogs fasted at least 24 hours before each experiment.

As there are variations in the reaction to histamine not only in different dogs, but in one and the same animal at various times, it was decided to follow the

[2] Parke Davis and Company.

procedure of Gray and his collaborators (9) and to take into final consideration only double histamine experiments. During the first period of the experiment, urogastrone was administered intravenously followed 10 minutes later by a subcutaneous injection of histamine. The gastric juice was then collected at 10 minute intervals for a duration of 70 minutes and titrated for free and total acidity. Three hours later histamine alone was injected and the gastric juice was again similarly obtained and titrated. This constituted the second period of the experiment. In a number of instances the above periods were reversed.

Urogastrone was administered more frequently in the first period of the double histamine experiments because, as will be seen below, when two experiments with histamine alone were performed on any one day and on the same animal it was found that the second injection of histamine produced a smaller secretion than the first in more than half of the studies.

Differences in secretion of ± 10 per cent or less between the two periods of the experiment were considered insignificant as showing neither stimulation nor inhibition.

Results. In 28 double experiments with histamine alone on gastric fistula and pouch dogs 11 gave a more abundant volume of secretion after the first injection than after the second, 8 produced a greater flow of juice after the second and 9 showed no significant differences between the two collections. The average difference in output of free HCl expressed in millequivalents (mE) between the response to the first and second introduction of histamine was less than 10 per cent.

In accordance with previous reports urogastrone prepared from normal dogs inhibited gastric secretion in the majority of experiments. Diminution of the volume of gastric juice occurred in 31 out of 47 experiments or 66 per cent, augmentation in 7 or 15 per cent, no important change in 9 or 19 per cent. Figures 1 and 2 show the inhibitions and stimulations of the volume-secretion at 10 minute intervals for a period of 70 minutes following administration of different urine extracts, as compared to the 100 per cent line representing control experiments with histamine alone. These are the averages of 4 to 23 experiments for each of two dogs (one gastric fistula and one pouch). The secretion curve after introduction of urogastrone from normal dogs is for the most part below the control line. The acidity was also reduced after urogastrone injection but it did not always correspond to the diminished flow of gastric juice. In table 1, the means of the total output of free HCl in mE are shown after the introduction of urine extracts followed by histamine and the per cent of inhibitions and stimulations are indicated as compared to the controls with histamine alone. A statistical analysis consisting in the determination of the standard error of the mean is included. Doses of completely purified extract of 1–2 mgm. were employed.

The extract prepared from urine of oöphorectomized dogs was tested in 26 experiments. Inhibition of volume of gastric secretion compared to histamine alone on the same day occurred in 12 studies or 46 per cent, an increase in 9 or 35 per cent and in 5 or 19 per cent no significant differences were obtained. In figure 1, injection of oöphorectomized dogs' extract produces an initial volume-

inhibition followed by a marked stimulation, thus the *most abundant secretion* is delayed as compared to that after introduction of normal urine extract or histamine alone. However, in figure 2 the volume-secretion is diminished with the exception of the first few minutes in relation to the control line and similar to the inhibition provoked by normal dogs' urine extract. Reduced quantities of gastric juice usually but not always coincided with lower acidity of the samples. In table 1, the line corresponding to the oöphorectomized dogs' urine extract shows no inhibition of the output of free HCl in mE from an average of 26 experiments as compared to the controls, but a slight stimulation (11 per cent). The difference of the response to histamine plus normal urine extract and histamine plus oöphorectomized urine extract was tested for significance by standard procedures.

TABLE 1

Statistical analysis of gastric secretion

EXTRACTS OF DOGS' URINE	TOTAL OUTPUT OF FREE HCL IN GASTRIC JUICE AFTER EXTRACT PLUS HISTAMINE ADMINISTRATION				DIFFERENCE OF MEANS IN mE AS COMPARED TO EFFECT OF NORMAL EXTRACTS	STANDARD ERROR OF THE DIFFERENCE OF THE MEANS	CRITICAL RATIO	PROBABILITY OF DIFFERENCES BY CHANCE IN 100
	Mean mE	Per cent inhibition as compared to control	Per cent stimulation as compared to control	Standard error of the mean				
Normal	4.02 (47 expts.)	25		0.573				
Oöphorectomized	6.0 (26 expts.)		11	1.19	1.98	1.32	1.5	15
Thyroidectomized plus oöphorectomized	4.0 (27 expts.)	13		0.71	0.02	0.912	0.02	50
Hypophysectomized	5.95 (58 expts.)		20	0.71	1.93	0.912	2.11	4

The critical ratio of 1.5 indicates that such a difference would be expected to occur by chance fifteen times in one hundred such experiments.

If, in addition to the ovaries the thyroids were removed, the same inhibitory effect was produced as from extracts of normal dogs (table 1, figs. 1 and 2). Inhibition of volume-secretion occurred in 17 out of 27 experiments or 63 per cent, increase in 5 or 18.5 per cent and no difference in the remaining 5 or 18.5 per cent. The critical ratio related to the difference of the means of the total output of HCl proved to be extremely low (0.02), thus indicating no difference as compared to the effect of normal urine extract. Taking into consideration the diminution of urogastrone after oöphorectomy alone, one is inclined to assume that the thyroid gland may inhibit the formation of inhibitory agents. Experiments are now under way to investigate this phase of the problem.

The extracts prepared from the urine of two hypophysectomized dogs inhibited

gastric secretion in only 6 out of 58 or a little over 10 per cent of the studies. But in 37 experiments or 64 per cent there was a marked increase in the volume of gastric juice, while in the remaining 15 or 26 per cent no significant difference was found. In figures 1 and 2, the dotted line related to volume-secretion of gastric juice after injection of hypophysectomized urine extract is the only one consistently above the control line with the exception of the first 15 minutes when the

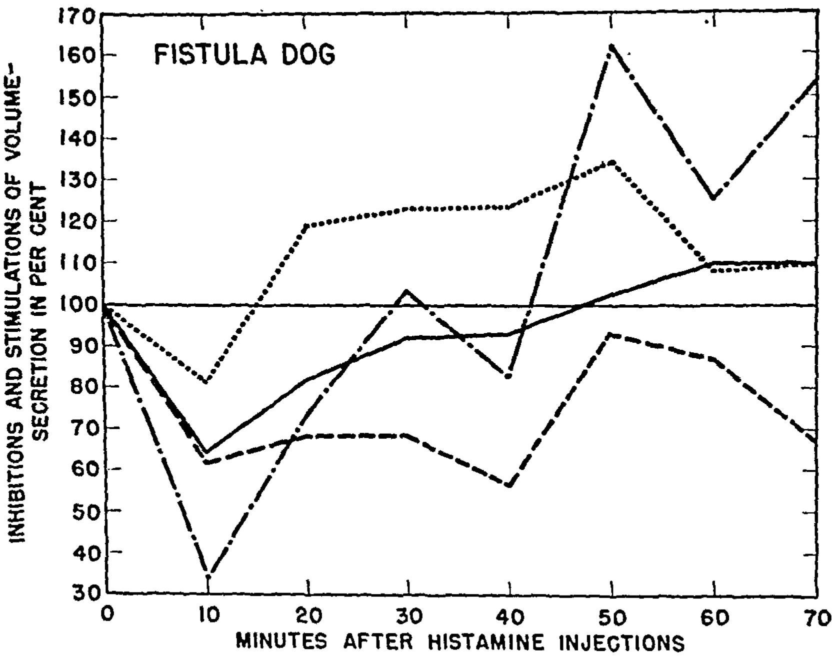

Fig. 1. Fistula dog. Volume-secretion curves resulting from intravenous injections of different kinds of dogs' urine extracts (urogastrone) on gastric secretion.

100% line = Histamine control: Secretion after histamine injections alone based on average of 23 experiments.

——————— Secretion after histamine injection and normal dog's urine extract. Average of 14 experiments.

– – – – – – – Secretion after histamine injection and thyroidectomized plus oöphorectomized dog's urine extract. Average of 6 experiments.

—— – —— – —— Secretion after histamine injection and oöphorectomized dog's urine extract. Average of 5 experiments.

· · · · · · · · · · · · Secretion after histamine injection and hypophysectomized dog's urine extract. Average of 9 experiments.

variations are the greatest, thus indicating an increase of the secretion. Variations in the acidity did not always correspond strictly to the changes in volume of gastric secretion. Therefore, although the mE of HCl output were increased in the identical number of experiments (37 out of 58), the number in which diminution or equality occurred were different. The augmentation of acid output amounted to 20 per cent (table 1). Quantities of 1–3 mgm. of the extract were employed in these studies. When larger doses were administered (5–10 mgm.) the increase in output of free HCl in mE rose to 27 per cent. The differ-

ence between the means of the response to histamine plus normal urine extract and histamine plus hypophysectomized dogs' urine extract was also tested for significance by standard procedures, which revealed that such a difference would be expected to occur by chance only four times in one hundred such experiments (table 1).

Thus, the normal dog has in its urine a gastric secretory inhibitor. However, when the pituitary gland is removed, the dog's urine contains very little, if any,

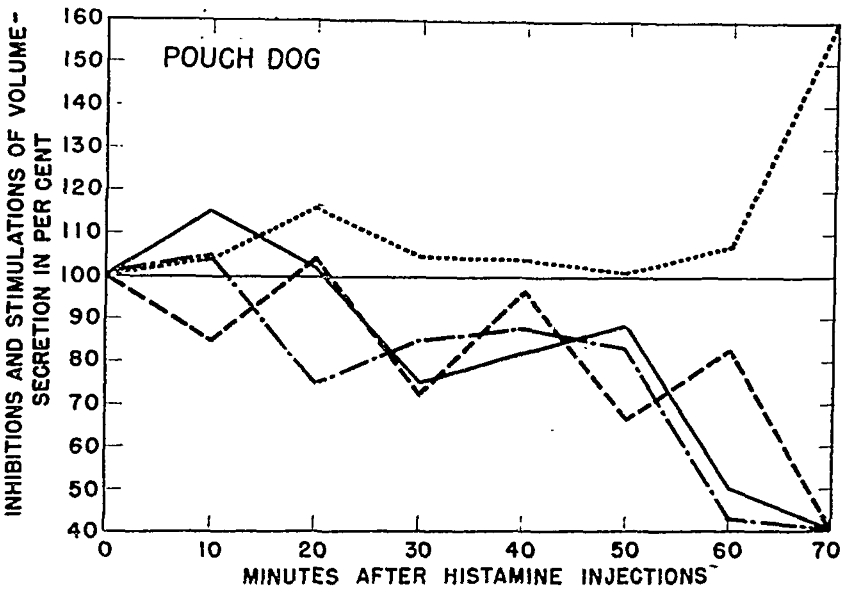

Fig. 2. Heidenhain pouch dog. Volume-secretion curves resulting from intravenous injections of different kinds of dogs' urine extracts (urogastrone) on gastric secretion.
100% line = Histamine control: Secretion after histamine injections alone based on average of 13 experiments.

——————— Secretion after histamine injection and normal dog's urine extract. Average of 5 experiments.

— — — — — — Secretion after histamine injection and thyroidectomized plus oöphorectomized dog's urine extract. Average of 6 experiments.

—— - —— - —— Secretion after histamine injection and oöphorectomized dog's urine extract. Average of 4 experiments.

. . . . Secretion after histamine injection and hypophysectomized dog's urine extract. Average of 9 experiments.

of the gastric secretory depressant. When such an extract is injected into pouch or fistula dogs, an increase in the quantity of gastric juice is noted in the majority of experiments.

Discussion. The results reported in this paper indicate that urogastrone obtained from normal urine and recognized as a gastric secretory inhibitor is also present when both the thyroids and ovaries are removed. There appears to be less urogastrone when the ovaries alone are extirpated. However, if the pituitary is removed the depressant is markedly diminished, if at all present, and in a large majority of experiments there was an actual increase in gastric secretion following administration of the urine extract prepared from the hypophysecto-

mized animals. This increase raises the question whether with a disturbance or removal of the pituitary gland, factors come into greater play which augment the action of gastric secretory stimulants. Some confirmation of the relationship of the hypophysis to gastric secretion may be inferred from the recently published report of Ungar (15) concerning the inhibition of histamine release by a pituitary-adrenal mechanism in the blood of normal rabbits mixed with peptone in vitro.

These experimental studies are closely related to some clinical observations on the rôle that the gonad mechanism plays in human peptic ulcer. Pregnancy has a decisive ameliorating effect, whereas the menopause aggravates the conditions (8). Persons suffering from ulcers not infrequently show endocrine abnormalities (8). Before puberty, though rare, peptic ulcer exhibits no sexual difference in frequency (8, 19). Urine extracts of women, both normal and pregnant, have a definite prophylactic, therapeutic and "immunizing" effect on Mann-Williamson ulcer in dogs (11, 8, 16, 17). It may be that in the female the mutual interrelations between the pituitary and ovaries during the active sex life and particularly at the time of pregnancies, favor the formation of factors protecting against the development of ulcers. The greater frequency in men may be due to a poorer development of these agents.

It is premature to conclude from the data herein presented that the pituitary gland is playing a decisive rôle in the regulation of the ulcer protective factors present in urine, since the relationship between urogastrone (the anti-acid factor) and anthelone (the anti-ulcer factor) is still uncertain. However, our studies appear to indicate that the hypophysis is somehow related to urogastrone excretion.

Normal urine contains among other factors pituitrin, which also inhibits gastric secretion (18). Gray et al. (10) however, differentiated pituitrin from urogastrone by a comparison of their effects on gastric secretion and motility, urine excretion and blood pressure. They came to the conclusion that the two principles are different entities. Because of the marked differences in their effects on gastric motility, urine excretion and blood pressure (10), it is probable that urogastrone does not contain pituitrin. It might be reasoned therefore, that the elimination of pituitrin from the urine by hypophysectomy does not explain the different effect on gastric secretion obtained with extracts from normal and hypophysectomized dogs.

SUMMARY

1. Extracts prepared from the urine of normal dogs (urogastrone) inhibit gastric secretion and decrease the acidity of gastric juice in the majority of the studies.

2. Urogastrone made from thyroidectomized plus oöphorectomized dogs exerts nearly the same inhibitory influence as urogastrone procured from normal animals. However, when the ovaries alone are removed, the depressing effect of the extract is less marked than that of normal urogastrone.

3. Hypophysectomized dogs do not produce urogastrone in adequate amounts to diminish gastric secretion, as is the case with urogastrone from normal dogs. On the contrary, urine extracts of such animals increase the quantity and the acidity of the gastric juice secreted after histamine injection.

4. A statistical analysis of the experimental data supports the conclusion that the pituitary gland plays a rôle in the formation or excretion of urogastrone.

Grateful acknowledgment is made to Dr. Gabriel Steiner for his examination of the hypothalamic regions of the brain and the decalcified bases of the skull. Also to Dr. A. C. Ivy of Northwestern University and his staff for their assistance in the statistical analysis of the data contained in this paper and to Doctor Thorstad and others of Harper Hospital for valuable surgical assistance in the early phases of this study.

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Addendum

Additional studies were recently performed with an urine extract prepared from another series of dogs hypophysectomized by the transtemporal instead of the transbuccal method. When this extract was administered during the first period of the double histamine experiment in all but one of 15 experiments an increase of the total output of free HCl in mE was noted (average 137 per cent stimulation) as compared to the controls. However, when the extract was administered in the second period in another 15 double histamine experiments, an increase was noted in 4 studies, a decrease in 8 and no significant change in 3, the average not differing more than ±10 per cent from the controls (Federation Proceedings, vol. 6, no. 1, p. 140. March 1947). The results will be presented in a subsequent communication.

THE PLASMATIC COFACTOR OF THROMBOPLASTIN: ITS ADSORPTION, WITH PROTHROMBIN AND FIBRINOGEN, BY ALUMINA AND TRICALCIUM PHOSPHATE GELS

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During the past few years increasing attention has been given to the structure of prothrombin and several conflicting views have arisen. Quick (9) in 1943 postulated the existence of two factors, designated as components A and B. The former decreased in human oxalated stored plasma, while component B decreased in avitaminosis K and after dicumarol poisoning. It could moreover be removed from plasma by adsorption with aluminum hydroxide. Several investigators, particularly Lavergne and Lavergne-Poindessault (3), and Loomis and Seegers (4) disagree with the concept of a dual nature of prothrombin and they explain the decrease of prothrombin activity in stored plasma as due to an alteration of fibrinogen.

The present study was begun with the purpose of standardizing the technique of adsorbing prothrombin with aluminum hydroxide (6) and with calcium phosphate, $Ca_3(PO_4)_2$ (1). It soon became apparent that the study had to include a quantitative estimation of the fibrinogen removed by these adsorbents and also an inquiry as to the nature and action of the plasma factor which disappears on storage.

Adsorption of Prothrombin and Fibrinogen by Aluminum Hydroxide and Tricalcium Phosphate. Reagents. Two-tenths mole aluminum hydroxide suspension. This stock solution was analyzed gravimetrically after heating a fixed amount to constant weight.

Two-tenths mole tricalcium phosphate stock suspension was used.

Technique for adsorption experiment. A measured quantity of either aluminum hydroxide or tricalcium phosphate was transferred to a test tube and then centrifuged. The supernatant water was poured off and the tube drained. A measured volume of oxalated plasma was then added thoroughly mixed with the adsorbent, and incubated at 37°C. for 10 minutes. The adsorbent was removed by high centrifugation in an angle centrifuge. The clear plasma was used for the studies recorded in tables 1 and 2.

Determination of prothrombin time. The one-stage method of Quick (8) was used.

Determination of fibrinogen. The technique given in Quick's Monograph was followed except that a photoelectric colorimeter was used.

Treatment of plasma with aluminum hydroxide affected the prothrombin time (as measured by Quick's method) and partially removed fibrinogen. When a

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TABLE 1

The effect of increasing concentration of aluminum hydroxide on the prothrombin time and on the adsorption of fibrinogen from oxalated dog, rabbit and human plasma

	FIBRINOGEN GM. PER 100 CC. PLASMA	PERCENTAGE OF FIBRINOGEN DECREASE WITH CONCENTRATION OF ALUMINUM HYDROXIDE							
		0.0	0.001 M	0.005 M	0.01 M	0.025 M	0.05 M	0.02 M	0.5 M
Dog	0.230		10.3	15.0	11.5	12.2	20.0	59.9	100
Rabbit	0.294		9.0	13.2	14.2	19.6	33.0	74.2	
Man	0.314		10.2	14.2	17.4	26.0	38.0	72.0	
		Prothrombin time in seconds							
Dog		5.7	6.6	16.2	40.0	70.0	150.0	No clot	
Rabbit		5.8	7.1	15.0	46.0	133.0	128.0	No clot	
Man		12.3	17.4	47.6	158.0	225.0*	320.0	No clot	

The values given for fibrinogen and for prothrombin time are averages from 6 to 11 specimens of plasma.

* Several specimens of human plasma failed to clot after treatment with as low as 0.025 M $Al(OH)_3$.

TABLE 2

The effect of increasing concentration of calcium phosphate on the prothrombin time and on the adsorption of fibrinogen from oxalated human and dog plasmas

HUMAN	PROTHROMBIN TIME (SEC.) (CONCENT. $Ca_3(PO_4)_2$ USED)					PERCENTAGE OF FIBRINOGEN DECREASED (CONCENT. $Ca_3(PO_4)_2$ USED)			
	0.005 M	0.01 M	0.015 M	0.02 M	0.2 M	0.015 M	0.02 M	0.16 M	0.2 M
1	70	no clot	370	no clot	no clot		11.8		32.2
2	45	no clot	600	no clot	no clot		12.7		30.3
3	185	205	380	no clot	no clot		5.6		36.2
4	34	113	no clot	no clot	no clot		1.5		28.3
5	46	415	no clot	no clot	no clot		0.0		26.2
6	50	205	no clot	780	no clot		6.1		32.0
Average	71	113—no clot	370—no clot	780—no clot	no clot		6.2		30.8
DOG									
1	33	230	230	no clot	no clot	6.4	0.0	24.5	40.5
2	25	233	280	no clot	no clot	14.4	9.7	30.5	19.3
3	25	265	no clot	no clot	no clot		7.8		46.0
4	30	no clot	no clot	no clot	no clot		0.0		28.8
5	43	no clot	no clot	no clot	no clot		30.2		54.0
6		no clot	no clot	no clot	no clot		1.5		
7		no clot	no clot	no clot	no clot		13.6		
Average	31.2	230—no clot	230—no clot	no clot	no clot	10.4	8.9	27.5	37.7

small amount of alumina was used (0.01M) for the treatment of fresh human plasma, the prothrombin time was 158 seconds and the loss of fibrinogen was 17 per cent. With higher amounts of alumina, completely incoagulable plasma could be obtained, but such plasma lost 60 per cent to 70 per cent of its fibrinogen.

By using tricalcium phosphate instead of aluminum hydroxide, it is possible to obtain human and dog plasmas that do not clot, yet have lost only 6.2 per cent to 8.9 per cent of their fibrinogen. The latter reagent is therefore preferable to aluminum hydroxide.

Action of tricalcium phosphate on plasma from dicumarolized animals. It is interesting to note that 192 hours after the injection of a single dose of dicumarol, when the prothrombin time of the plasma had returned presumably to the normal (6.5 sec.) the response to tricalcium phosphate was strikingly different from that

TABLE 3

The effect of increasing calcium phosphate concentration on the prothrombin time of plasmas from dogs injected with dicumarol

CONCENT. $Ca_3(PO_4)_2$ USED	PROTHROMBIN TIME (SEC.)				
	HOURS AFTER DICUMAROL†				
	24	48	96	120	192
0.0	12	18	16.5	15	6.5
0.0001M	11	17			
0.0005M	13:5	21	16		
0.001M	16.5	35	85	65	7
0.002M		300	no clot	no clot	19
0.003M		no clot			55
0.004M					305
0.005M*					no clot

* The normal value for 0.005M is 31 seconds.

† A single dose of dicumarol, 5 mgm./kgm. of body weight was injected intravenously.

of normal plasma (table 3). For example: normal dog plasma treated with 0.005M calcium phosphate has a prothrombin time of 31 seconds, yet the plasma of the dog recovering from dicumarol poisoning did not clot after such treatment.

While the prolongation of the prothrombin time is due to a decrease of prothrombin (2), (7), a return to complete normality after dicumarol cannot apparently be conclusively established by the prothrombin time determination. If one assumes that the amount of calcium phosphate needed to obtain a noncoagulable plasma is in direct proportion to the amount of prothrombin present, one must conclude that even though the dog recovering from dicumarol poisoning shows a normal prothrombin time, he actually has less than 100 per cent prothrombin present in his blood.

Prothrombin Factors A and B Studied with Aluminum Hydroxide. Prothrombin time recovery in stored human plasma on addition of fresh fibrinogen-free human alumina plasma. Fresh human plasma was treated with 0.01M $Al(OH)_3$, then

all the fibrinogen was clotted by the addition of a small quantity of thrombin. A mixture of fresh fibrinogen-free alumina plasma and stored human plasma was made. The results confirm those obtained by Quick (10). It shall be noted that the prothrombin times obtained are always shorter than those of the normal fresh human plasmas. For example:

	Prothrombin time seconds
Stored human plasma (7 days)	29
Mixture of stored plasma with fresh fibrinogen-free alumina plasma (human)	10

TABLE 4

The recovery of the prothrombin time of stored human plasma by means of fresh human plasma treated with varying amounts of aluminum hydroxide

Prothrombin time (sec.)

STORED PLASMA NOT TREATED	STORED PLASMA + FRESH ALUMINA PLASMA (CONC. $Al(OH)_3$ USED)			
	0.2M	0.05M	0.01M	0.005M
30	25	14.5	12	12
24	26	15	13	13
43	40	15		
17	19	15	12	
30	27	16	13	

Concentration $Al(OH)_3$ used

STORED PLASMA NOT TREATED	STORED PLASMA		FRESH PLASMA		PROTHROMBIN TIME
	0.005M	0.01M	0.005M	0.01M	
0.1 cc.					43
	0.1 cc.				117
			0.1 cc.		45
	0.05 cc.		0.05 cc.		28
		0.1 cc.			220
				0.1 cc.	149
		0.05 cc.		0.05 cc.	49

As a control to establish that the added thrombin became inactivated, the fresh fibrinogen-free alumina plasma was added to fresh plasma. No clot resulted.

The activity of fresh alumina plasma on stored plasma can therefore not be attributed to its fibrinogen. Further proof for this is that fresh oxalated dog plasma heated to 56°C. to coagulate the fibrinogen still retains its activity to normalize stored plasma. Human plasma, however, on heating loses its activity. The results recorded in table 4 affirm that it is possible to normalize the prothrombin time of stored human plasma by the addition of fresh human plasma treated with aluminum hydroxide. At the same time they prove that the recovery is related to the concentration of $Al(OH)_3$ used. If the fresh human plasma is treated with 0.05M aluminum hydroxide, the recovery is not complete, and with 0.2M alumina there is no recovery at all.

If one accepts that prothrombin is composed of two factors, it must be further assumed that both factors are adsorbed by 0.2M $Al(OH)_3$. Only when the fresh plasma is treated with aluminum hydroxide weaker than 0.05M is the prothrombin time of the stored plasma completely recovered.

The theory that prothrombin is composed of two factors was based on the assumption that $Al(OH)_3$ adsorbed only component B, and that storage destroys only component A. The normalization of the stored plasma was explained as the result of the union of the A remaining in the alumina plasma with the B which remains unaltered in the stored plasma. However, if both the fresh and the stored human plasmas are treated with 0.01M or 0.005M aluminum hydroxide

TABLE 5

Titration of antithrombins and antithromboplastins in stored human plasma

THROMBOPLASTIN CONCENTRATION	PROTHROMBIN TIME (SEC.)*	
	Dil. with fresh human plasma	Dil. with 6 days' human stored plasma
100	12	12
75	12	11
50	14	12
25	17	14
10	21	22
5	28	26
2.5	40	32
1.25	62	55

* The prothrombin times were checked against fresh human plasma.

AGE OF PLASMA	PROTHROMBIN TIME	THROMBIN DILUTIONS (TIME SEC.)			
		F.S.	1/5	1/10	1/20
days					
0	12	3.5	24	55	65
2	18	3.5	16	30	43
6	29	5		58	110

(thus adsorbing component B in both plasmas) it is still possible at least partially to restore the prothrombin time (table 4) by combining the two.

These experiments affirm that fresh human plasma treated with aluminum hydroxide in proper concentration is able to normalize stored human plasma. They also prove that the factor responsible for the recovery can not belong to prothrombin, and that the factor is adsorbed along with prothrombin by aluminum hydroxide when the concentration of the latter is increased to 0.2M.

It might be postulated as Quick (9) has done that dog and rabbit blood contains several times the concentration of this factor present in human blood. This would explain the very short prothrombin times obtained in these animals, which Quick interprets as due to a higher concentration of prothrombin.

Action of Concentrated Fibrinogen-Free Dog Alumina Plasma. The material

was prepared as follows: fresh dog plasma was treated with 0.1M $Al(OH)_3$ which produced a plasma that did not clot. The fibrinogen was removed with human thrombin. This plasma deprived of prothrombin and fibrinogen was dialyzed in the refrigerator overnight in cellophane bags against 50 per cent dextrin, and a concentration of about six times the original was obtained. By the addition of only 0.02 cc. of this concentrate to 0.1 cc. of stored human plasma +0.1 cc. of thromboplastin +0.1 cc. of calcium chloride it was possible to reduce the pro-

TABLE 6

Effect of the addition of the cofactor of thromboplastin and of the fresh and stored human plasmas on the prothrombin time of progressive dilutions of human plasma, including results on dicumarol dogs

CONCENTRATION OF FRESH HUMAN PLASMA** IN PERCENTAGE	PROTHROMBIN TIME (SEC.)					
	Fresh	Fresh + 0.03 cc. conc. TBP cofactor*	Fresh + 0.03 cc. fresh undil.	Fresh + 0.03 cc. stored undil.	Fresh + 0.03 cc. fresh undil. + 0.03 cc. cofactor	Fresh + 0.03 cc. stored undil. + 0.03 cc. cofactor
100	12	11		10		9
90	12	11				
80	12	11				
70	12	12				
60	13	13				
50	15	14.5		10		9
40	16	14.5				
30	17	14				
20	17.5	13				
10	23.5	13		14		11
5	50	50	17	19	17	13
Dogs injected with dicumarol (Prothrombin time after 24 hrs.)						
100	12	13				7.5
10	46	45	16	11		

* Prepared from dog plasma. Conc. TBP cofactor = concentrated cofactor of thromboplastin.

** Diluted with distilled water.

thrombin time of the stored plasma consistently from 60 to 7.5 or 8 seconds. This experiment shows that the substance does not dialyze.

When the concentrated treated plasma (heated or unheated) is added to stored human plasma, the prothrombin time is reduced much below the normal of 12 seconds. This effect is not obtained when the concentrate is added to fresh human plasma, but fresh plasma treated with $Al(OH)_3$ can reduce the prothrombin time of stored plasma to 10 seconds (table 6).

The fact that fresh plasma treated with $Al(OH)_3$ reduces the prothrombin time of stored plasma more than of fresh plasma suggests that a change in the fibrinogen may perhaps occur. Lyons (5) has demonstrated the existence of two kinds of fibrinogen which he calls A and B, and states that the latter is easily obtainable from stored plasma. It thus appears that stored plasma gradually loses a factor

necessary for coagulation, for which the name plasmatic cofactor of thromboplastin is suggested; and at the same time fibrinogen undergoes a qualitative change which renders it more easily coagulable.

Antithromboplastin and Antithrombins in Stored Human Plasma. The presence of antithromboplastin was studied by making various dilutions of thromboplastin using fresh and stored human plasma as diluents, and after incubation, testing the mixture for thromboplastic activity on fresh human plasma. From the results as shown in table 5 it can be concluded that no antithromboplastins are demonstrable in plasma stored for 6 days.

The antithrombins of fresh and stored plasmas were studied with serial dilutions of fresh human thrombin following the technique of Quick. The results recorded in table 5 demonstrate that no antithrombins developed in human plasma stored for 48 hours during which period the prothrombin time increased from 12 to 18 seconds. In fact, the response to serial dilutions of thrombin was better for the 48 hour old plasma than for the fresh plasma. Assuming that the delayed prothrombin time is due to the decreasing concentration of the plasmatic cofactor, these findings are taken to indicate that: 1, the cofactor must act in the thromboplastin-prothrombin phase, rather than in the thrombin-fibrinogen phase; 2, the fibrinogen present is more readily coagulable than in fresh plasma; and 3, no antithrombins have developed in 48 hours. After six days, the findings with thrombin dilutions may well be attributed to the presence of antithrombins.

The Relation of Prothrombin and the Plasmatic Cofactor to the Prothrombin Time. It becomes apparent that two factors determine the rapidity of the prothrombin time: 1, prothrombin itself (the factor which Quick calls component B) and 2, the plasmatic factor which the writer believes is necessary for the action of thromboplastin. This affects the reliability of results obtained with any technique in which one of these is not previously stabilized or supplied in excess. A means of transforming the thromboplastin cofactor into a constant in the prothrombin method of Quick is the previously described use of a special concentrate prepared from dog plasma. The influence of only 0.03 cc. of this concentate on the prothrombin time of normal fresh human plasma in increasing dilutions with distilled water (table 6) demonstrates the importance of this control.

From these experiments it may be judged that no more than 10 per cent of prothrombin is necessary to obtain times of 13 seconds.

In order to establish whether a given delayed prothrombin time is due to a lack of prothrombin or to a lack of cofactor, it is necessary to study the influence of the concentrate on the plasma diluted 1/10 and 1/20. If it brings about a normalization of the prothrombin time in these dilutions the conclusion must be that the prolonged prothrombin time is due to a decrease in the cofactor in the plasma and not to a decrease in the prothrombin. The concentrate did not produce changes in the prothrombin times of dogs injected with dicumarol; the deficiency here is in the prothrombin itself.

In the case of the dogs injected with dicumarol, the difference between the

normalization produced by the addition of fresh undiluted human plasma (46 to 16 sec.) and that produced by the addition of stored undiluted human plasma (46 to 11 sec.) must be attributed to the difference in the quality of the fibrinogen provided by the stored plasma, because the dicumarolized dog plasma supplies cofactor in excess.

SUMMARY

By means of a standardized technique with aluminum hydroxide and tricalcium phosphate in various concentrations it has been possible to establish the proportions in which the prothrombin and fibrinogen are adsorbed from human, dog, and rabbit plasmas. At the same time it has been shown that the plasma factor which disappears on storage and which has been designated plasmatic cofactor of thromboplastin, does not belong to prothrombin and is also adsorbed by $Al(OH)_3$ but to a less extent and is only completely removed when a 0.2M concentration of aluminum hydroxide is used. A concentration of this cofactor is described, involving the total elimination of prothrombin and fibrinogen. The ability of this substance to normalize stored human plasma is demonstrated, and its place in the control of prothrombin time determination is discussed.

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THE EFFECT OF CARBOHYDRATE FEEDING ON THE OUTPUT OF URINARY AMINO ACID[1]

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In 1856 Hoppe (1) reported that carbohydrate ingestion lowered the nitrogen excretion of dogs. This finding was later corroborated by Lusk (2) by a reverse experiment on himself which demonstrated that the withdrawal of 350 grams of carbohydrate from the diet at two levels of N intake (20.55 and 9.23 grams per day) increased his urinary N output by 4 grams above the usual values in both instances. This protein sparing action of the carbohydrates was also found to occur in the fasting dog (3) and man (4). The practical implications of this phenomenon were demonstrated by the experiments of Keller (5), Orgler (6), and Rosenstern (7) which demonstrated that the N-retention of the infant from a given amount of protein could be greatly augmented by increasing the carbohydrate content of the diet. More recently Larson and Chaikoff (8) reported that in dogs the occurrence and degree of retention of total N, ammonia N, urea N and inorganic S effected by a single feeding of carbohydrate was inversely related to the time elapsing between the ingestion of the extra carbohydrate and the daily meal and was confined to the 12 hour period immediately succeeding the administration of carbohydrate.

In contrast to the clarity of the end result the metabolic mechanics of the protein sparing action of the carbohydrates remain obscure. It is generally assumed that carbohydrates spare proteins: first, by preventing the formation of glucose from amino acids; secondly, by supplying the total caloric needs in the form of a fuel which is mobile and readily available, and thirdly, by providing chemical groupings which are easily convertible into amino acids. Experiments with phlorhizinized dogs reveal that the phenomenon is not wholly due to an isodynamic replacement, since in these test animals ingested glucose spares protein despite the fact that the glucose producing the effect is being rapidly excreted (8). The direct involvement of the metabolism of the amino acids as a part of the sparing effect was first indicated by the human experiment of Folin and Berglund (10) which disclosed that a single dose of 200 grams of glucose given subsequent to the noon meal caused a transient but marked decrease not only in the amino N, non-protein N, and urea N levels of the blood but also of the amino N, urea N, and total N of the urine. Subsequently, Brunschwig and his co-workers (11) presented evidence in humans that the ingestion of amino acids without glucose tends to lead to a greater output of nitrogen in the urine than when glucose is added. Also, the existence of some common pathways in

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the intermediary metabolism of carbohydrates and the amino acids is suggested by the studies of Luetscher (12) who found that the high plasma and urinary amino acid levels of patients with diabetes mellitus could be returned to normal levels by insulin therapy.

The work of these previous investigators suggested to us that further information on the protein sparing mechanism induced by the carbohydrates could be secured by a study of the urinary amino acid excretion patterns prevailing after the administration of carbohydrate (13). The results of these experiments which are presented here showed that in the infant and adult the urinary output of total N, amino N and tryptophane was reduced below normal values in the 3 hour period following the administration of single doses of various carbohydrates. Inasmuch as the output of the 8 other amino acids measured was not significantly affected, it appears that the sparing mechanism involves tryptophane and components of the undetermined fraction of the urinary amino acids.

Experimental. Since it has been shown that the protein sparing action of the carbohydrates can be affected at all levels of N intake (2) and that the effect is maximal in the 3 hour period following the administration of the carbohydrate (8), it was decided to dispense with the measurement of nitrogen balances and to secure our data from the morning urine passed in the 3 hours succeeding the ingestion of carbohydrate, a time known to constitute a period of fairly constant nitrogen excretion provided uniform urine volumes are obtained. Preliminary experiments in the adult human led to the adoption of the following procedure. Prior to a standard breakfast consisting of 200 cc. of orange juice, two slices of buttered toast and one cup of coffee with cream and sugar, the subjects voided and discarded all of the night urine at 8:00 a.m. One hour after the standard breakfast, the zero hour specimens were collected and the subjects were given by mouth the carbohydrate dose dissolved in 240 cc. of water. In order to maintain constant urine flow 120 cc. of water were also given at the end of the succeeding hours of the experiment. The urines, preserved with alcoholic thymol, were collected hourly and analyzed individually or as pools for the period of study. The control experiments were similarly performed except that no carbohydrate was fed.

The infants studied in this investigation were maintained on a formula, consisting of 235 cc. of evaporated milk, 30 grams of dextri-maltose and water to make 840 cc., which was given in five 170 cc. feedings at the following times: 6 and 10 a.m., 2, 6, and 10 p.m. For a determination of the N retention effect of carbohydrates, 15 grams of sucrose were added to the 10 a.m. feeding. In these experiments the urine excreted during the intervals between 6 a.m., 10 a.m. and 2 p.m. feedings were collected separately by means of urinary adapters for each period and immediately submitted to analysis.

Results. The effects of carbohydrate feeding on tryptophane and other nitrogenous excretory products were reported briefly elsewhere (13). These experiments disclosed that the nitrogen and tryptophane sparing action of the various sugars could be consistently demonstrated only when optimal output of urine was effected. By varying the total water intake for the 3 hour period from

100 to 960 cc., it was found that the output of tryptophane and other urinary metabolites became maximal and fairly constant at an intake of 480 cc. or more of water providing the urine volume was greater than 85 per cent of the intake.

The typical data collected in table 1 which were obtained when 480 cc. of water were given indicate the effect of carbohydrate on the tryptophane is transient and is not detectable in the fourth hour following the ingestion of extra

TABLE 1

Effect of sucrose feeding on urinary output of total tryptophane

SUBJECT	SUCROSE ADMINISTERED	HOURS AFTER SUCROSE INGESTION					TOTAL OUTPUT*
		0	1	2	3	4	
	grams	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
A, male, 70 kgm.	None	3.5	3.1	4.7	4.1	3.6	11.9
	25	3.9	0.0	0.0	0.0	3.0	0.0
J, female, 60 kgm.	None	3.8	4.2	4.0	4.1	4.4	12.3
	25	3.0	4.5	0.0	0.0	4.7	4.5

* This includes amount of total tryptophane excreted in 3 hour period following administration of sucrose.

TABLE 2

Effect of single feeding of different sugars on the nitrogen excretion of the normal adult human

The subjects breakfasted at 8:00 a.m. and were fed the various sugars at 9:00 a.m. All measurements were made on specimens collected from 9:00 a.m.–12 noon.

SUBJECT	A, MALE, 70 KGM.			F, FEMALE, 50 KGM.			I, FEMALE, 60 KGM.		
Sugar fed	Total N	Amino N	Tryptophane	Total N	Amino N	Tryptophane	Total N	Amino N	Tryptophane
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
None*	1616	39.9	11.8	1453	37.4	12.1	1684	29.9	18.2
25 gm. glucose*	1272	29.0	0.6	996	23.2	5.6	1564	28.3	7.8
50 gm. glucose							1505	29.4	6.1
25 gm. sucrose*	1209	29.3	0.0	1081	30.8	3.9	1477	32.1	4.6
50 gm. sucrose				1285	31.9	4.1			
25 gm. fructose	1435	24.9	0.0						
25 gm. arabinose	1450	36.5	14.8						

* Average results of 3 experiments.

sugar. In several instances, however, the effect lasted through the fourth and fifth hours after the administration of sucrose. The effects of single doses of different amounts of various sugars on the total N (14), amino N (15), and tryptophane (16) output of the adult under the conditions noted are shown in table 2. It will be seen from these results that *a*, the ingestion of glucose and fructose as well as sucrose causes the output of total urinary tryptophane to fall below the control levels; *b*, the feeding of 50 grams of these carbohydrates caused no greater decrease in the tryptophane excretion than the 25 gram dose,

and *c*, in general the carbohydrate induced fall in urinary tryptophane was paralleled by a drop in amino N and total N values. Attention is called to the fact that the drop in tryptophane excretion does not account for the total fall in amino N output, so that the levels of amino acids other than those measured must be affected by the carbohydrate ingestion. It is of interest that, unlike the total N output, the amino N and tryptophane levels of the urine were not effected by the administration of the unphysiological carbohydrate, arabinose.

The findings in the study of the infant parallel those of the adult (table 3). Moreover, the analytical data show that the retention effected by the extra carbohydrate feeding in period 2 is compensated for the most part by a rise in the output of nitrogen containing metabolites in period 3. Similar observations were made in the dog by Larson and Chaikoff (8).

TABLE 3

Effect of single feeding of sucrose on the nitrogen excretion of the infant

SUBJECT	EXPERIMENT NO.	URINARY METABOLITES	NORMAL DIET			NORMAL DIET WITH 15 GRAMS SUCROSE SUPPLEMENT GIVEN AT 10:00 A.M.		
			Period 1 6–10 a.m.	Period 2 10 a.m.–2 p.m.	Period 3 2–6 p.m.	Period 1 6–10 a.m.	Period 2 10 a.m.–2 p.m.	Period 3 2–6 p.m.
		mgm.						
Golden, 5 mos., 4.7 kilos; 840 cc. of formula daily in five feedings	1	Total N	311	409	432	319	311	373
		Amino N	3.4	4.2	3.4	4.6	3.4	6.6
		Tryptophane	4.7	6.5	7.2	5.8	2.1	7.2
	2	Total N	387	418	388	296	172	704
		Amino N	13.7	16.4	11.2	8.1	4.2	18.2
		Tryptophane	4.7	4.7	4.1	4.6	1.4	8.8
Harris, 4 mos., 4.8 kilos; 840 cc. of formula daily in five feedings	3	Total N	264	206	430	279	118	443
		Amino N	6.1	6.8	6.7	8.3	3.0	14.6
		Tryptophane	7.0	6.4	6.3	4.5	1.1	5.7
	4	Total N	209	423	421	281	278	406
		Amino N	7.6	15.3	14.3	11.2	10.7	19.1
		Tryptophane	4.2	4.7	7.7	5.2	3.0	6.1

COMMENT. The findings reported here amply confirm in man the observations of Larson and Chaikoff (8) on the effect of single doses of carbohydrate on the urinary nitrogen output of the dog. Our failure to note any effect of the carbohydrates on the inorganic of other sulfur constituents of the urine which they found to occur in the dog may be due in part to differences in the metabolic requirements of the sulfur amino acids of man and the fur-bearing animals which are suggested by the recent report of Cox and collaborators (17).

The observation that the mechanism of the carbohydrates involves the essential amino acid tryptophane, which is considered to be neither ketogenic nor glucogenic (18), lends support to the prevailing view that the phenomenon is not

due to a simple transposition of energy either in the form of calories or chemical radicals. The transient nature of the observed effect suggests that the biochemical reaction is an immediate rather than a secondary one as is the case in the tryptophane sparing effect of carbohydrates observed in the rat by Krehl and co-workers (19) which they believe to arise from an improved intestinal synthesis afforded by some protein-carbohydrate relationships. Inasmuch as the method employed for the estimation of tryptophane appears to measure both free and bound tryptophane our data fail to disclose which urinary tryptophane fraction is reduced by the administration of extra carbohydrate. Information on this point might reveal in which metabolic phase, anabolic or catabolic, the sparing action was affected. Pending the development of suitable techniques for the estimation of the two urinary tryptophane fractions and the amino acids of the undetermined amino N moiety, it would seem unwise to theorize on the reported observations.

SUMMARY

It has been observed that the administration of single doses of various carbohydrates to adult humans and infants induces a transient fall in the level of total urinary tryptophane which is associated with a decrease in amino N and total N excretion. The output of the other chemically measurable amino acids seemed to be affected by the carbohydrate ingestion. The significance of these findings is discussed.

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THE INDUCTION OF LACTATION DURING PREGNANCY IN RABBITS AND THE SPECIFICITY OF THE LACTOGENIC HORMONE[1]

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In 1941 the authors (1) suggested that copious lactation could be initiated only when *a*, there is a well developed mammary gland present, and *b*, when there is a high lactogen content in the pituitary. Lobule-alveolar mammary growth had been shown to be complete by mid-pregnancy (for review see Turner, 2), while numerous studies of the lactogen content of the pituitary revealed that it remains low during pregnancy in most animals studied but increases sharply, by several hundred percent, soon after parturition (for review see Meites and Turner 3, 4, 5,) and Hurst and Turner (6)). As a result of these findings it was postulated that the principal reason copious lactation was not initiated during pregnancy or pseudopregnancy was because of insufficient secretion of pituitary lactogen.

Attempts to initiate lactation with lactogenic extracts during pregnancy in guinea pigs, rats, rabbits and goats usually resulted in resorption of the embryos or abortion of the fetuses, with the consequent onset of lactation (7, 8, 9, 10). In no instance was lactation reported to precede abortion or resorption of the embryos. In the rabbit, De Fremery and Denekamp (8) found that daily subcutaneous injections of lactogen for five days during the first, second or third 10 days of gestation (a rabbit has a gestation period of approximately 30 days) invariably resulted in abortion or resorption. While these experiments demonstrated that the mammary glands of pregnant animals were capable of secreting milk, they seemingly substantiated the view of those who believed that some inhibitory factor or factors prevented the harmonious co-existence of pregnancy and lactation. Since this view was contradicted by the fact that pregnancy and lactation do go simultaneously in many species without serious detriment to either process (1), it seemed to the writers that the termination of pregnancy induced by lactogenic extracts was probably due to some impurity associated with the available lactogen preparations.

In 1942 Lyons (11) demonstrated for the first time the direct action of lactogen on the mammary gland. He was able to induce a localized lactation in one small sector of the rabbit mammary gland by the intraductal injection of as little as 1–3 I.U. of lactogen. This technique provided an opportunity to inject an amount of lactogen small enough, it was hoped, to initiate a localized lactation without terminating pregnancy.

[1] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1045.

Experiment 1. Thirty-one pregnant and pseudopregnant New Zealand White rabbits were injected with various doses of lactogen by the intraductal route. Pseudopregnancy was usually induced by a single intravenous injection of 200 I.U. of chorionic gonadotrophin.[2] All lactogen doses were given in either single or multiple injections, in ½ ml. volumes, beginning on the 10th or 15th days of pregnancy or pseudopregnancy, and the animals were killed 5 days later. Four

TABLE 1

Mammary sector reactions to varying doses of lactogenic hormone during pregnancy and pseudopregnancy

NO. OF RABBITS	CONDITION OF DOES	LACTOGEN UNITS INJECTED PER DOE*	DAY KILLED	STATE OF FETUSES	NO. OF POSITIVE RESPONSES
5	Pregnant	5 I.U. on days 15, 16, 17, 18	20	Alive	4+
3	Pregnant	10 I.U. on day 15	20	Alive	3+
2	Pregnant	10 I.U. on day 15	Permitted to go to parturition	Normal litters	2+
3	Pregnant	5 I.U. on days 10, 11, 12, 13	15	Involuting	2+
2	Pregnant	50 I.U. on day 10	15	Involuting	2+
2	Pregnant	10 I.U. on day 10	Permitted to go to parturition	Normal litters	2+
3	Pseudo-pregnant	5 I.U. on days 15, 16, 17, 18	20		3+
3	Pseudo-pregnant	10 I.U. on days 15, 16, 17, 18	20		3+ general lactation
3	Pseudo-pregnant	10 I.U. on day 15	20		3+
3	Pseudo-pregnant	5 I.U. on days 10, 11, 12, 13	15		3+
2	Pseudo-pregnant	50 I.U. on day 10	15		2+

* Only one nipple was injected in each rabbit.

pregnant rabbits were permitted to continue their pregnancies to term. Only one teat was injected per rabbit, but as each teat usually had 6 excretory milk ducts no attempt was made to inject the lactogen into the same duct when multiple injections were given over a 4-day period.

RESULTS AND DISCUSSION. It will be seen that with two exceptions lactation was induced in all the pregnant and pseudopregnant rabbits (table 1). In agree-

[2] We wish to thank Ayerst, McKenna and Harrison Ltd., Rouses Point, N. Y., for the chorionic gonadotrophin, "A.P.L."

ment with Lyons (11), a considerable variation in individual response was found. On the whole a single injection of 10 I.U. of lactogen into one duct of a teat was more effective than 4 injections of 5 I.U. daily into different ducts of the same teat. When the injections were begun on the 15th day, milk could usually be expressed from the nipple by the 18th day. No remarkable difference in lactational response was noted between the pregnant and pseudopregnant rabbits.

Pregnancy was not terminated in any of the 10 rabbits which received injections beginning on the 15th day, and the two rabbits which were permitted to live beyond the 20th day dropped normal litters on the 31st day. Lactation had been induced in 9 of these rabbits.

In rabbits injected with total dosages of 20 to 50 I.U. of lactogen beginning on the 10th day of pregnancy, resorption of the embryos occurred in the five rabbits killed on the 15th day. Lactation had been initiated in four of these animals. In the remaining two rabbits, which had each received a single injection of 10 I.U. of lactogen, a small amount of milk could be expressed from the injected teats by the 15th day. These does were permitted to continue in what was hoped was still a normal pregnant state, and they both dropped viable litters on the 31st and 32nd days.

It is noteworthy that three of the rabbits in whom resorption occurred following the injections begun on the 10th day of pregnancy received no more lactogen (20 I.U.) than five rabbits injected beginning on the 15th day and in whom the fetuses lived. Since it has been claimed that the pituitary factor which maintains luteal function during the first half of pregnancy (luteotrophin) is lactogen, it seems rather contradictory that the injection of a small dosage (0.66 mgm.) of this hormone should terminate pregnancy. It is possible, however, that the embryos are more sensitive to any contaminant in the lactogen during the earlier period since Hammond and Marshall (12) reported that fixation of the embryos and formation of the placentae take place in the rabbit on the 10th or 11th day of pregnancy. It is also noteworthy that the ovaries of the rabbits in whom resorption took place contained many blood follicles and were highly vascularized, showing that an intense stimulation had occurred. However, it is not known whether this was associated with the termination of pregnancy.

These data are believed to confirm the previously expressed view that lactation is not initiated during most of pregnancy, or during pseudopregnancy, primarily because insufficient lactogen is being secreted by the pituitary. A localized lactation was induced in most of the pregnant rabbits reported here without interfering with pregnancy (see fig. 1 for type of lactation induced). It is believed that if a sufficient dosage of truly pure lactogenic hormone were injected systemically, a generalized lactation could be initiated without terminating pregnancy. That the two processes of lactation and pregnancy are not antagonistic is known from the fact that they can be present simultaneously in many species, including the mouse, rat, rabbit, goat, cow and human.

Experiment 2. Bergman and Turner (13) defined the specificity of lactogenic hormone as its ability to initiate lactation in intact animals with properly developed mammary glands. In support of this thesis they demonstrated in pseudo-

pregnant rabbits that while lactation could be induced with lactogenic hormone which was relatively free of other pituitary hormones, pituitary extracts relatively free of lactogen could not induce lactation. The identity of lactogen with the pigeon crop proliferating factor was also established by showing that its lactation inducing ability was directly related to its pigeon crop proliferating potency (13, 14). Later Lyons (11) provided positive proof of the direct action of lactogen on the mammary epithelium.

Since Lyons had shown that a localized lactation could be induced in the rabbit mammary gland with a small intraductal injection of lactogen, it was decided to apply this technique to determine whether other hormones were also capable of inducing lactation. The following hormones were injected into separate nipples of each of 3 pseudopregnant rabbits: lactogen, whole pituitary extract, thyrotrophin, thyroxine and adrenal cortical hormone.[3] Each of these hormones was given in a single 0.5 ml. intraductal injection into the same 5 teats

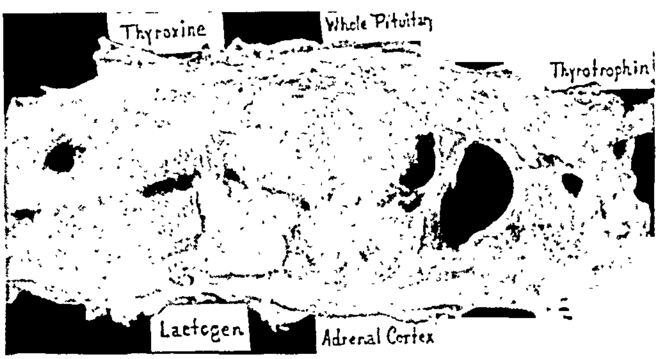

Fig. 1. Effect of different hormones injected intraductally into individual teats of a pseudopregnant rabbit. This whole mount of the mammary gland shows the local lactational response induced with lactogen. The lesser response obtained with whole pituitary extract had largely faded by the time this picture was taken (about 48 hrs. post mortem).

in each rabbit. All injections were made under ether anesthesia on the 15th day of pseudopregnancy, and the animals were killed 5 days later.

The results are summarized in table 2. It will be seen that only those mammary sectors which received lactogen or whole pituitary extract came into lactation. Figure 1 provides a striking illustration of the type of localized lactation induced in one small sector of the mammary gland by a single intraductal injection of extract containing lactogen. Paradoxically, the lactation induced by the whole pituitary extract did not equal that induced by the lactogenic hormone, despite the fact that the former presumably contained as much lactogen as the latter. However, the whole pituitary extract was prepared and assayed in 1941, and even though it had been stored as a dry powder, it may have lost

[3] We are indebted to the Schering Corp., Bloomfield, N. J., for the lactogen; the Upjohn Co., Kalamazoo, Mich., for the adrenal cortex extract; the British Drug Houses, Ltd., London, for the thyroxine; and to Dr. A. J. Bergman, formerly of this laboratory, for the whole pituitary and thyrotrophic extracts, and for their assays.

some of its original potency. In no case did the thyrotrophin, thyroxine or adrenal cortical hormone induce lactation. An examination of the ovaries of these rabbits revealed that multiple ovulation and formation of corpora lutea occurred in each case.

These experiments, although limited in number, are believed to demonstrate that there is no basis for the claim that other hormones, particularly those controlling or emanating from the adrenals or thyroids, have an equal claim with lactogen to be considered as specific lactation inducing hormones. Only lactogen is capable of acting directly on the mammary epithelium to induce lactation. Other hormones are believed to be essential or supplemental to lactation only insofar as they influence important metabolic processes necessary to the mobilization of milk precursors in the blood, i.e., salt and water metabolism; carbohydrate, fat and protein metabolism; metabolic rate, etc. This explains why the pituitary and adrenals are essential for normal lactation, and why whole pituitary extracts are more effective than lactogen alone in augmenting established lactation.

TABLE 2

Mammary sector reactions to 5 different hormones injected into 5 separate nipples of each of 3 rabbits

HORMONE INJECTED	DOSAGE OF HORMONE		AVERAGE REACTION PER GLAND SECTOR*
	mgm.	Approx. units	
Lactogen	0.33	10 I.U.	+++
Whole pit. ext.**	2.20	10 I.U. lactogen 6.5 chick units thyrotrophin 2.3 chick units gondadotrophin 0.2 chick units adrenotrophin	++
Thyrotrophin**	5.00	55 chick units	—
Thyroxine	0.20		—
Adrenal cortex	20,000.00 Approx.	25 dog units	—

* The Gardner-Turner (15) method of rating rabbit mammary responses was used.

** The assays of these hormones were made according to the methods described by Bergman and Turner (16).

SUMMARY

Lactation was induced in pregnant and pseudopregnant rabbits by injecting relatively small amounts of lactogenic hormone intraductally into localized sectors of the rabbit mammary gland. Either single or four daily injections were made directly into one nipple of each rabbit. Nine of ten rabbits injected beginning on the 15th day of pregnancy came into lactation, usually by the 18th day, without disturbing the pregnant state. Resorption of the embryos occurred in 5 rabbits given 20 to 50 I.U. of lactogen beginning on the 10th day of pregnancy, but not in two does given a single injection of 10 I.U. of lactogen. Pseudopreg-

nant rabbits responded similarly to pregnant rabbits when given the same dosage of lactogen. Better lactational responses were obtained when injections were begun on the 15th than on the 10th day of pregnancy or pseudopregnancy.

These experiments are believed to demonstrate that the principal reason lactation is not normally initiated during pregnancy or pseudopregnancy is because of an insufficient secretion of lactogen by the pituitary. For the first time lactation has been initiated in pregnant animals by exogenous lactogen administration without terminating pregnancy.

In another experiment attempts were made to determine whether hormones other than lactogen could induce localized lactation by the intraductal injection technique. On the 15th day of pseudopregnancy three rabbits received single injections into 5 nipples of each of the following hormones: lactogen, whole pituitary extract, thyrotrophin, thyroxine and adrenal cortical hormone. Only the mammary sectors which received lactogen and whole pituitary extract came into lactation. This is believed to show that only lactogen has the specific property of acting directly on the mammary epithelium to induce lactation.

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THE EFFECTS OF THE PITUITARY GROWTH AND ADRENOCORTICOTROPIC HORMONES ON THE URINARY GLUCOSE AND NITROGEN OF DIABETIC RATS[1]

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The production of diabetes mellitus by the injection of suitable crude anterior hypophyseal extracts into normal animals (1, 2, 3) is a well recognized phenomenon. Equally well known is the fact that hypophysectomy lessens the severity of the diabetes in a depancreatized animal (4) and that suitable crude hypophyseal extracts, when injected into such a doubly operated animal, reinvoke diabetes mellitus in its full severity. Whether these effects of crude extract are due to their content of one of the known hormones, to some unknown factor, or to a synergistic effect between some of the known hormones is not clear. Relatively little work has been done with chemically or biologically pure preparations. Anderson, Marx and Fong (5) have reported that biologically pure growth hormone increased the glycosuria of partially depancreatized rats supplied with sucrose in their drinking water. Ingle, Li and Evans (6) used chemically and biologically pure adrenocorticotropic hormone and produced glycosuria and increased nitrogen excretion in normal rats forced fed a high carbohydrate diet. It is the purpose of this paper to report the effects produced in diabetic rats by chemically and biologically pure growth and adrenocorticotropic hormones.

Materials and methods. In these experiments male rats of the Long-Evans strain weighing approximately 200 grams were used. Diabetes mellitus was produced by the intraperitoneal injection on each of two successive days of alloxan monohydrate (Eastman) at a dose level of 200 mgm. per kgm. body weight. Animals with persistent severe diabetes exhibiting a daily glycosuria of more than 3.5 grams were selected for study. During metabolic study the animals were maintained in individual metabolism cages and were fed a restricted, weighed amount of the stock[2] diet so adjusted that all diet was consumed each day. Thus each animal reported in this paper was maintained on a constant food intake through the entire experimental period. The animals were fed at the same time each day and were weighed prior to feeding. Urine was collected daily under toluene and was analyzed for glucose and total nitrogen by the Shaeffer-Somogyi (7) method and the micro-Kjeldahl procedure. Yeast fermentation studies demonstrated that the daily excretion of non-fermentable

[1] Aided by grants from the Research Board of the University of California, the James Foundation Grant of the Medical School, and the Rockefeller Foundation of New York City.

[2] The diet consisted of ground whole wheat 68½ per cent, fish oil 5 per cent, casein 5 per cent, alfalfa leaf meal 10 per cent, fish meal 10 per cent, and sodium chloride 1½ per cent.

reducing substances was never more than 80 mgm. per day and so only the data for total reducing substances are reported in this paper.

The hormone preparations used were prepared according to the previously published methods (8, 9) and in most instances were injected subcutaneously three times a day. The hormone was injected for 5 or 6 days and before its administration the glucose and nitrogen excretion was followed for a control period of from 10 to 17 days. When insulin was given it was in the form of protamine zinc insulin and was administered once daily subcutaneously. Statistical analysis according to the methods of Fisher (10) was made for each animal, comparing the significance of the change between the control and injection

TABLE 1

The effect of 3 mgm. per day of growth hormone on the glycosuria of diabetic rats

RAT NO.	FOOD INTAKE	INSULIN DOSE PER DAY	MGM. URINARY GLUCOSE PER DAY: Control period before growth hormone	Growth hormone treatment period	p	Mean change from the control period
	gms.					
B 8261	20	None	5420 ± 130*(15)†	6670 ± 260(6)	<0.01	+1250
B 8854	20	None	5820 ± 220(15)	5940 ± 240(6)	0.70	+120
B 8261	20	None	7440 ± 220(17)	8150 ± 200(6)	0.05	+710
B 8255	15	None	5120 ± 90(13)	5630 ± 230(6)	0.015	+510
W 5538	16	None	5720 ± 80(13)	6080 ± 400(6)	0.20	+360
G 5531	16	None	5790 ± 90(13)	6970 ± 230(6)	<0.01	+1880
B 7504	18	None	6710 ± 70(14)	6530 ± 430(6)	0.50	−180
B 7047	18	None	6560 ± 100(13)	7120 ± 340(6)	0.03	+560
B 7060	18	1.5 units	2330 ± 400(10)	2310 ± 340(6)	>0.90	−20
BH 6882	18	1.5 units	1660 ± 170(10)	3410 ± 350(6)	<0.01	+1750
BH 7088	18	1.5 units	220 ± 60(10)	750 ± 220(6)	<0.01	+530
B 7081	18	1.5 units	500 ± 120(10)	465 ± 140(6)	>0.90	−35
B 7040	18	1.5 units	1060 ± 210(10)	1090 ± 160(6)	>0.90	+30

* Standard deviation of the mean.
† The figure in the parentheses indicates the number of days' observation.

period. A p value of 0.01, indicating one chance in one hundred that the observed difference could be due to random variation, was taken as significant.

RESULTS. In tables 1 and 2 are presented the data which summarize the experiments in which growth hormone was administered at a level of 3 mgm. per day. It will be seen that in each case in which exogenous insulin was supplied and that in five of the eight experiments in which no exogenous insulin was supplied there was a significant nitrogen retention as shown by the decrease in urinary nitrogen excretion. The effects upon the degree of glycosuria were not so consistent, as there was a significant increase in glycosuria in only four of the thirteen experiments. Ten additional experiments were done in which only 1 mgm. of growth hormone was administered per day; in five of these experiments insulin was also given and in all five there was a definite nitrogen retention. In the other five animals no insulin was administered but there was

nevertheless a definite nitrogen retention in four of the five cases. None of the ten animals receiving the smaller amount of growth hormone exhibited a significant change in glycosuria.

In tables 3 and 4 are presented the data summarizing the experiments in which 3 mgm. of adrenocorticotropic hormone were given per day. It will be seen that in every case, regardless of whether exogenous insulin was given, there was a marked increase in the degree of glycosuria with a p value of less than 0.01 in ten of the twelve cases. There was an associated increase in nitrogen excretion in each instance, being statistically highly significant in all but five of the animals. Five additional experiments were done in which only 1 mgm. of adrenocorticotropic hormone plus insulin was administered per day. In none of these

TABLE 2

The effect of 3 mgm. per day of growth hormone on the urinary nitrogen excretion of diabetic rats

RAT NO.	FOOD INTAKE	INSULIN DOSE PER DAY	MGM. URINARY NITROGEN PER DAY			
			Control period before growth hormone	Growth hormone treatment period	p	Mean change from the control period
	gms.					
B 8261	20	None	382 ± 5(15)	392 ± 5(6)	0.25	+10
B 8854	20	None	423 ± 15(15)	356 ± 13(6)	0.02	−67
B 8261	20	None	406 ± 8(17)	362 ± 15(6)	<0.01	−44
B 8255	15	None	340 ± 15(13)	306 ± 8(6)	0.15	−34
W 5538	16	None	346 ± 5(13)	250 ± 12(6)	<0.01	−96
G 5531	16	None	369 ± 9(13)	249 ± 12(6)	<0.01	−120
B 7504	18	None	454 ± 7(14)	355 ± 18(6)	<0.01	−99
B 7047	18	None	426 ± 7(13)	381 ± 13(6)	<0.01	−45
B 7060	18	1.5 units	351 ± 8(10)	266 ± 19(6)	<0.01	−85
BH 6882	18	1.5 units	356 ± 12(10)	284 ± 14(6)	<0.01	−72
BH 7088	18	1.5 units	379 ± 7(10)	272 ± 15(6)	<0.01	−107
B 7081	18	1.5 units	292 ± 15(10)	234 ± 12(6)	<0.01	−58
B 7040	18	1.5 units	364 ± 8(10)	252 ± 16(6)	<0.01	−112

animals was there a change in either nitrogen excretion or glycosuria that was judged to be significant.

With both hormones the body weights of the animals reflected the changes in nitrogen excretion, gain in weight occurring when nitrogen was retained and loss in weight occurring when nitrogen excretion was increased. The maximum rate of gain occurred in those animals receiving both growth hormone and insulin, reaching an average of 7 grams per day for a 5-day period in one animal.

Discussion. It seems quite clear that the effect of growth hormone at both levels of administration and either with or without administration of additional insulin was to promote nitrogen storage, as there was a significant decrease in nitrogen excretion in nineteen of twenty-three experiments. Thus in the diabetic rat growth hormone has the same effect upon nitrogen metabolism as it does in the normal rat. It seems equally clear that at the 1 mgm. level there was no significant effect on glycosuria, while at the 3 mgm. level it apparently enhanced glycosuria in approximately one-third of the cases.

Thus growth hormone is not diabetes enhancing with respect to its effects upon nitrogen excretion, but may be with regard to glycosuria when adminis-

TABLE 3

The effect of 3 mgm. per day of adrenocorticotropic hormone on the glycosuria of diabetic rats. Values for glucose reported as milligram per day

RAT NO.	FOOD INTAKE	INSULIN DOSE PER DAY	MGM. URINARY GLUCOSE PER DAY			
			Control period before adrenocorticotropic hormone	Adrenocorticotropic hormone treatment period	p	Mean change from the control period
	gms.					
B 8255	20	None	6280 ± 90(15)	7180 ± 140(5)	<0.01	+900
B 5931	15	None	3650 ± 180(13)	4440 ± 500(5)	0.05	+790
B 5927	20	None	5960 ± 180(14)	7960 ± 350(5)	<0.01	+2000
W 5538	20	None	6050 ± 150(15)	7810 ± 400(5)	<0.01	+1760
G 5531	20	None	5980 ± 160(15)	7380 ± 300(5)	<0.01	+1400
B 8261	20	None	5420 ± 130(15)	7360 ± 190(5)	<0.01	+1940
B 8854	20	None	5820 ± 220(15)	7270 ± 260(5)	<0.01	+1850
G 8261	20	None	7440 ± 200(17)	8890 ± 70(5)	<0.01	+1450
B 8261	18	2.0 units	2380 ± 195(13)	3850 ± 620(6)	<0.01	+1470
B 8854	18	2.0 units	950 ± 140(13)	5400 ± 1000(6)	<0.01	+4450
G 8261	18	2.0 units	1510 ± 280(13)	6080 ± 620(6)	<0.01	+4570
G 7018	18	1.5 units	680 ± 100(10)	1170 ± 290(6)	0.05	+490

TABLE 4

The effect of 3 mgm. per day of adrenocorticotropic hormone on the urinary nitrogen excretion of diabetic rats. Values for nitrogen reported as milligram per day

RAT NO.	FOOD INTAKE	INSULIN DOSE PER DAY	MGM. URINARY NITROGEN PER DAY			
			Control period before adrenocorticotropic hormone	Adrenocorticotropic hormone treatment period	p	Mean change from the control period
	gms.					
B 8255	20	None	476 ± 4(15)	486 ± 12(5)	0.30	+10
B 5931	15	None	327 ± 8(13)	344 ± 17(5)	0.30	+17
B 5927	20	None	442 ± 8(14)	499 ± 30(5)	0.01	+57
W 5538	20	None	466 ± 8(15)	530 ± 23(5)	<0.01	+64
G 5531	20	None	488 ± 7(15)	509 ± 21(5)	0.20	+21
B 8261	20	None	382 ± 5(15)	460 ± 22(5)	<0.01	+78
B 8854	20	None	423 ± 15(15)	510 ± 27(5)	<0.01	+87
G 8621	20	None	406 ± 8(17)	479 ± 13(5)	<0.01	+73
B 8261	18	2.0 units	275 ± 9(13)	326 ± 24(6)	0.02	+51
B 8854	18	2.0 units	269 ± 7(13)	394 ± 27(6)	<0.01	+125
G 8261	18	2.0 units	296 ± 3(13)	366 ± 15(6)	<0.01	+76
G 7018	18	1.5 units	332 ± 6(10)	363 ± 16(6)	0.03	+31

tered at the higher dose level. Since the alloxanized rats with which we worked undoubtedly retained some functioning islet tissue, the fact that growth hormone promoted nitrogen storage even though no exogenous insulin was supplied cannot be construed to indicate that this effect does not require the presence of insulin, or that growth hormone would have the same effect in a completely de-

pancreatized animal without exogenous insulin. Since the nitrogen retaining effect of growth hormone occurred even in the presence of an increase in glycosuria it is highly unlikely that this effect on nitrogen metabolism could be mediated by stimulation of the islets of Langerhans to increase their insulin output.

The effect of adrenocorticotropic hormone at the 1 mgm. level was not significant while at the 3 mgm. level it increased both glycosuria and nitrogen excretion. Thus adrenocorticotropic hormone is clearly diabetes enhancing and "anti-insulin" in its effects on both nitrogen and glucose excretion in the diabetic rat, in that it produces the characteristic effects of insulin deficiency. It is also to be noted that the effects of growth hormone on nitrogen excretion were demonstrated more consistently than were its effects on glycosuria while for adrenocorticotropic hormone the reverse was true.

It is of interest to note in the experiments with adrenocorticotropic hormone in which no exogenous insulin was given that, on the average, the nitrogen excretion increased 51 mgm. per day while the glycosuria increased 1480 mgm. per day. If one accepts a D/N ratio of 3.65 as representing maximum glyconeogenesis from protein, the increase in nitrogen excretion would account for only 180 mgm. of the extra glucose excreted. Even if one assumed complete conversion of protein to glucose the extra protein broken down is still inadequate to account for the additional glycosuria. However, it is not necessary to assume gluconeogenesis from any non-carbohydrate source to explain the observed effects in these experiments, since the glycosuria never exceeded the dietary intake of pre-formed carbohydrate.

SUMMARY

1. Growth hormone produced nitrogen storage and adrenocorticotropic hormone produced nitrogen loss in rats with alloxan-induced diabetes, both with and without exogenous insulin.

2. Growth hormone occasionally produced an increase and adrenocorticotropic hormone consistently produced a marked increase of the glycosuria of rats with alloxan-induced diabetes, both with and without exogenous insulin.

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THE RELATION OF FIBRINOGEN TO THE COAGULATION FACTOR WHICH DIMINISHED IN STORED PLASMA

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In 1943 Quick (1) observed that the prolonged prothrombin time of stored oxalated human plasma could be restored to normal by the addition of fresh rabbit or dog plasma treated with $Al(OH)_3$ or by plasma from dicumarol poisoned animals. On the basis of these observations Quick postulated the existence of two components of prothrombin: A, the labile factor that disappears from stored plasma, and B, which is readily adsorbed by $Al(OH)_3$ and is diminished in all the known conditions of hypoprothrombinemia. Since Lavergne and Lavergne-Poindessault (2) claimed that alteration in the fibrinogen of stored plasma affected the prothrombin time, the action of fresh defibrinated rabbit plasma treated with $Al(OH)_3$ was tested on stored plasma. It was found that full restoration of prothrombin time occurred, which makes it obvious that the fibrinogen of stored plasma is not responsible for the delayed prothrombin time (3). In fact Honorato (4) has observed that the fibrinogen in stored plasma is actually easier to clot than that of fresh plasma.

Relatively little is known concerning the labile factor that is destroyed by storage. Recently Honorato (4) has been able to concentrate the agent in fresh dog plasma and has found that it is more stable to heat than either fibrinogen or prothrombin (component B). The rôle of the substance under discussion is not known. One of us (R. H.) believes it functions as a co-factor of thromboplastin, but to avoid confusion the agent will be referred to in this paper as the "labile factor." The report of Owren (5) on a hemorrhagic condition in which the prothrombin time is prolonged, thus simulating a true hypoprothrombinemia, but which is actually due to a deficiency of a substance in plasma which is similar to or identical with the labile factor, makes it imperative to study further the influence of the latter on the prothrombin time determination.

The effect on the prothrombin time obtained by adding human and rabbit fibrinogen to stored human plasma. The fibrinogen obtained from 3 cc. of fresh rabbit and human plasma (by adding 1.2 volumes of saturated sodium chloride to 1 volume of plasma) was redissolved in 2 cc. of stored human oxalated plasma. The following results were obtained:

Experiment 1

Prothrombin time* of stored plasma	35 sec.
Prothrombin time* of stored plasma + human fibrinogen	31 sec.
Prothrombin time of stored plasma	75 sec.
Prothrombin time of stored plasma + rabbit fibrinogen	15 sec.

* By the method of Quick using acetone dehydrated rabbit brain as the source of thromboplastin.

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It can be clearly seen that rabbit fibrinogen has a striking effect in decreasing the prothrombin time of stored plasma whereas human fibrinogen does not. In order to determine whether this is due intrinsically to the fibrinogen itself, or to an admixture of the labile factor and fibrinogen, the following experiments were done:

Experiment 2

1. Stored oxalated human plasma (Prothrombin time 75 sec.)	0.4 cc.
2. Stored oxalated human plasma + rabbit fibrinogen (Prothrombin time 15 sec.*)	0.1 cc.
Prothrombin time of mixture 18 sec.	

* From experiment 1.

Experiment 3

1. Stored oxalated human plasma (Prothrombin time 75 sec.)	0.1 cc.
2. Same as plasma mixture 2 of expt. 2 except defibrinated*	0.1 cc.
Prothrombin time of mixture 17 sec.	

* The stored human plasma with the dissolved rabbit fibrinogen was completely defibrinated with a small amount of thrombin and allowed to stand until all thrombin activity disappeared (as tested by mixing equal volumes of the defibrinated plasma with fresh oxalated plasma and obtaining no coagulation).

Obviously, the decrease in prothrombin time obtained by adding fibrinogen

TABLE 1

Thromboplastic potency of dehydrated rabbit brain and of lung (Maltine) on fresh and stored human plasma

		PROTHROMBIN TIME	
		Rabbit brain*	Lung (Maltine)
		sec.	*sec.*
Human plasma, fresh		12	16
Human plasma, stored I		53	35
Human plasma, stored II		64	35
Human plasma, stored III		55	35
Human plasma, stored IV		55	35
Human plasma, stored V		61	40
Human plasma, stored VI		62	34
Stored plasma	0.3 cc.		
Fresh human alumina plasma†	0.1 cc.	20	25
Stored plasma II	0.3 cc.		
Fresh human alumina plasma†	0.1 cc.	18	24
Stored plasma II	0.3 cc.		
Fresh human alumina plasma††	0.1 cc.	14	21

* Prepared according to the directions of Quick.
† Plasma treated with 0.01 M $Al(OH)_3$ (4).
†† Plasma treated with 0.005 M $Al(OH)_3$.

to stored oxalated plasma is not due to the fibrinogen itself but to a small amount of the "labile factor" which is carried along as a contaminant. This is clearly shown by the fact that the plasma to which the fibrinogen was added retained this accelerated prothrombin time even after the fibrinogen had been removed. Since rabbit plasma has a much higher concentration of the labile factor, it is logical to expect that rabbit fibrinogen should carry more of the contaminant than human fibrinogen, which is clearly brought out by experiment 1. Failure to recognize the possibility that fibrinogen may carry a significant quantity of the labile factor has led Loomis and Seegers (6) into the error of concluding that this factor (component A) is identical with fibrinogen.

The thromboplastic activity of dehydrated rabbit brain as contrasted with dried lung (Maltine)[2] *when tested on fresh and stored human plasma.* The experimental procedure and the results are clearly presented in table 1. It will be noted that lung thromboplastin is definitely inferior to rabbit brain when tested on fresh human plasma, but shortens the prothrombin time of stored plasma more than rabbit brain thromboplastin. These divergent results can readily be explained. Lung which is difficult to free of blood contains an appreciable amount of the labile factor, whereas brain is practically free and represents the purest thromboplastin thus far obtainable. The relatively shorter prothrombin time of stored plasma to which lung thromboplastin is added results not only from the thromboplastin but also from a partial restoration of the labile factor. When fresh alumina plasma is added to stored plasma, rabbit brain again shows greater thromboplastic potency since the amount of labile factor supplied overshadowed the amount in lung extract. The labile factor is to some extent adsorbed by $Al(OH)_3$ and it can be noted that when a smaller amount of the adsorbent is employed, a greater shortening of the prothrombin time occurs.

These observations have both theoretical and practical significance. It is doubtful whether a mixture of pure prothrombin, pure thromboplastin and calcium will react to form thrombin in the absence of the labile factor. It should again be emphasized that a lung extract can not be considered pure thromboplastin. Any tissue extract containing traces of blood will contain sufficient amounts of the labile factor to render the prothrombin determination inaccurate; and it is conceivable that a hemorrhagic diathesis such as reported by Owren might be missed should the thromboplastin contain sufficient quantity of the labile factor to cover the deficiency in the pathological blood.

SUMMARY

The addition of fibrinogen from fresh plasma added to stored human plasma partially restores the delayed prothrombin time due to the fact that fibrinogen contains a small amount of the labile factor that disappears on storage. Rabbit fibrinogen contains more of the factor than human plasma.

Thromboplastin prepared from lung is less potent on fresh plasma than that obtained from rabbit brain, but yields a shorter prothrombin time when added

[2] Kindly supplied by Dr. Ralph S. Overman.

to stored plasma—a result explainable on the basis that lung thromboplastin contains a considerable quantity of the labile factor as a contaminant.

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THE REVERSIBLE INACTIVATION OF PROTHROMBIN: A FACTOR RESPONSIBLE FOR ITS PARTIAL REACTIVATION

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Quick (1–4) has published data showing that prothrombin activity, as measured by the one stage method, is the result of the combination of two factors which he designated as prothrombin A and prothrombin B. The data on which this hypothesis was based have been confirmed by Oneal and Lam (5), by ourselves (6, 7), and partially by Loomis and Seegers (8). The latter authors, however, failed to find any alteration in the prothrombin content of plasma as the result of storage when determinations were made by the two stage method. They claim that the observation that there is a decrease in prothrombin activity in stored plasma, as determined by the one stage method, is due to a change in the reactivity of the fibrinogen and is not related to the prothrombin content of the plasma. They apparently overlooked the fact that in both our publications we stated that we added fibrinogen as a reagent to all the samples tested. They further state that "no conclusions concerning prothrombin concentrations may be drawn from work dealing with species heterogeneous mixtures of plasma", and that "in work with homologous species there is no sound evidence for a second prothrombin component."

In this paper we present data showing that even when materials from homologous species are used there is still strong evidence for some factor which influences prothrombin activity and which is not fibrinogen. The presence of this factor has been demonstrated in defibrinated plasma fractions and in serum. We do not consider this factor to be a component of prothrombin, as stated by Quick. The data indicate that some factor is essential for the activation of prothrombin and we are presenting suggestions as to the possible nature of this factor.

METHODS AND MATERIALS. All plasma fractions used in this study were prepared from a pool of fresh rabbit plasma. Since the lack of agreement in the experimental data obtained by various workers is at least partially the result of failure to duplicate experimental conditions, we feel it advisable to present in detail the methods used in preparing these factors.

Fibrinogen. Fibrinogen was prepared from fresh plasma according to the method described by Jaques (9). The purified product contained 250 mgm. of fibrinogen per 100 ml. This fibrinogen has been shown by electrophoresis to migrate as a single boundary at two separate pH values (10) and has consistently had at least 99 per cent clottable protein.

Plasma containing factor A. Two types of plasma containing factor A were used. One of these was obtained from a rabbit which had been given 5 mgm.

of dicumarol per kilo, by stomach tube, daily for a period of 7 days. The other was prepared by treating fresh plasma with aluminum hydroxide[1] as described by Quick (1). Each of these plasmas were used both before and after defibrination by the addition of $\frac{1}{10}$ its volume of thrombin[2].

Plasma containing factor B. Plasma containing factor B was obtained by adjusting fresh oxalated plasma to pH 10.5 (7) and allowing it to stand at room temperature for four hours. After readjusting to pH 7.4, a part of this plasma was defibrinated by adding thrombin. A second lot of the same plasma was defibrinated before the pH was adjusted to 10.5.

Prothrombin determinations. Prothrombin was determined by Quick's method (11). This was modified in two respects: 0.1 ml. of fibrinogen prepared as described by Jaques (9) was added to the plasma mixtures, and the thromboplastin suspension and calcium chloride solution were mixed in equal proportions; 0.2 ml. of this mixture then was added to the plasma-fibrinogen mixture and the clotting time measured from this point.

We have shown (7) that there is practically no change in prothrombin time over a fairly wide range of proportions of plasmas containing factor A and factor B. In studying the interaction of the various factor A and B preparations we have, therefore, used mixtures containing equal amounts of the two preparations. Prothrombin determinations were made on each of the A factor preparations, the B factor preparations, and on all possible combinations of these preparations. In order to determine whether heterogeneity of species was an important factor, all the A factor preparations were tested against a B factor preparation obtained from dog plasma, and some of the A and B combinations were tested using fibrinogen prepared from human plasma in place of the rabbit plasma fibrinogen.

Prothrombin times were determined on a series of dilutions of the normal rabbit plasma used in the preparation of the various fractions employed in these experiments. In order to maintain comparable conditions, 0.1 ml. of fibrinogen was also added to the test mixtures in this dilution series. The percentage of prothrombin present in the various samples tested was determined from the curve obtained for the dilution series.

RESULTS. The data obtained in these experiments are given in table 1, and the values for the dilution series on the normal plasma are shown graphically in figure 1. The percentage of prothrombin equivalent to the prothrombin times was read directly from the dilution curve for the samples containing only factor A or factor B. When mixtures of factor A and factor B were used, the B factor which we consider to be prothrombin is diluted 1:1. The prothrombin concentrations of these mixtures were, therefore, determined by reading the concentration equivalent to the prothrombin time and then multiplying this value by two.

[1] Wyeth's Amphogel, without flavor—This product conforms to the U.S.P. standards for aluminum hydroxide gel. We are indebted to Dr. Alfred Barol, Director of the Wyeth Institute of Applied Biochemistry, for supplies of this material, and for information regarding its properties.

[2] Parke, Davis and Company Thrombin Topical—1 ampoule being made up to a volume of 200 ml. with 0.15 N sodium chloride. We are indebted to Dr. Eugene C. Loomis for generous supplies of this material.

It is apparent from the data that there is a marked reduction in the prothrombin time by the one stage method when plasma containing factor A is mixed in equal proportions with plasma containing factor B. That this effect is not due to the nature of the fibrinogen present is obvious since the same active fibrinogen was present in all tests and since closely similar results were obtained with samples which differed only in the fact that one was defibrinated while the other was not.

It is also apparent that mixing plasma preparations from different species does not markedly alter the type of results obtained. There is actually a somewhat better restoration of prothrombin activity when plasma preparations from a single species are used.

TABLE 1

The increase in prothrombin activity in mixtures of factor A and factor B

	A PREPNS. ALONE		B-1		B-1D		B-2		B-3	
	sec.	%	sec.	%	sec.	%	sec.	%	sec.	%
Rabbit fibrinogen										
B prepns. alone			28	6	26	5	38	4	72	3
A-1	220	1	11	46	11	46	11	46	12	40
A-1D	136	1	13	36	12	40	11	46	15	28
A-2	300	1	17	22	16	26	15	28	19	18
A-2D	300	1	13	36	11	46	12	40	14	30
Human fibrinogen										
B prepns. alone					31	5	41	4		
A-1D	104	1			15	28	14	30		
A-2	300	1			17	22	17	22		

A-1—Plasma from a dicumarol treated rabbit.
A-1D—A-1 defibrinated.
A-2—Normal rabbit plasma treated with aluminum hydroxide.
A-2D—A-2 defibrinated.
B-1—Normal rabbit plasma kept at pH 10.5 for 4 hrs. and then neutralized to pH7.4.
B-1D—B-1 defibrinated.
B-2—Defibrinated rabbit plasma treated as B-1.
B-3—Dog plasma treated as B-1 but for 24 hrs.

In table 2 we present an experiment in which rabbit serum was tested for the presence of factor A. These tests were made approximately two hours after collecting the blood, and at that time the serum still had traces of prothrombin present. This observation is similar to that of Warner, Brinkhous and Smith (12) that dog serum contained 5 per cent of its original prothrombin three hours after the blood was collected. The serum contained no fibrinogen as shown by its failure to clot on the addition of thrombin, and no thrombin as shown by its failure to clot on addition of fibrinogen. However, when this serum was mixed with a B factor preparation, having a prothrombin time of 106 seconds, the prothrombin time of the mixture was 11 seconds.

Discussion. The data presented here demonstrate that prothrombin activity as measured by the one stage technique is the result of the combination of two factors. They also demonstrate that fibrinogen is not the factor responsible for the reactivation of stored plasma. Finally, they demonstrate that studies made on preparations from plasma of a single species give results essentially the same as those obtained when preparations from plasma of different species are used.

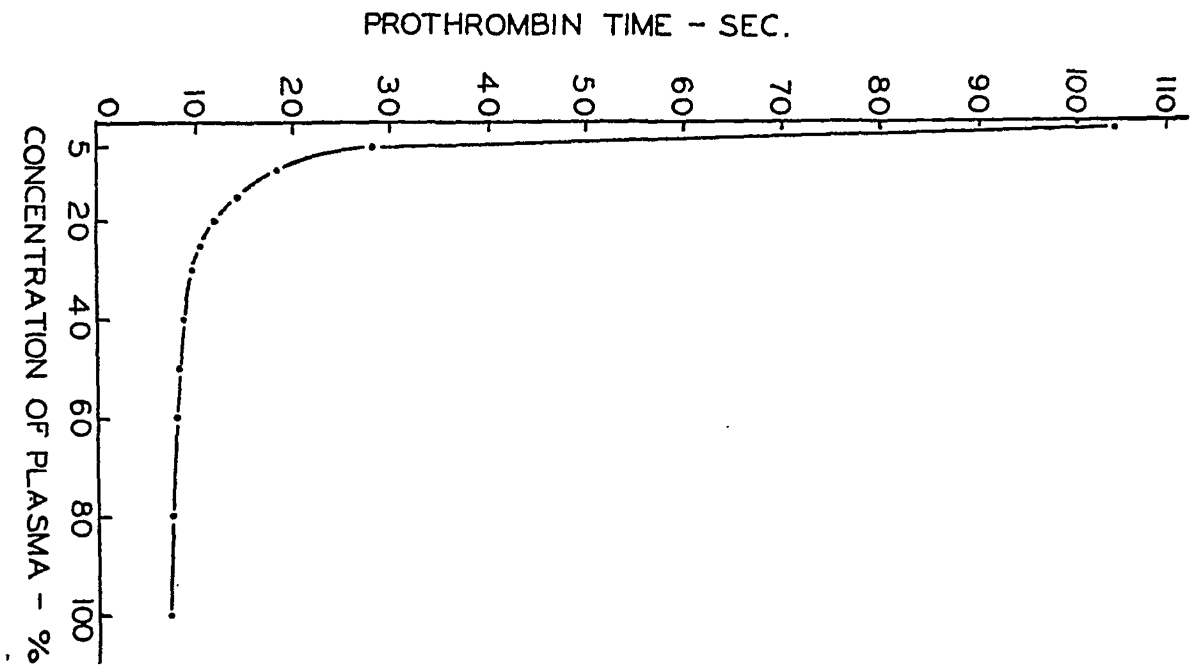

Fig. 1. Dilution curve of normal rabbit plasma.

TABLE 2

The A factor activity of rabbit serum

	PROTHROMBIN TIME
	sec.
Rabbit serum	84
B factor preparation	106
1 part serum + 1 part B factor	11

While we do not consider that the data regarding the interaction of the B factor and the A factor can be explained on the basis of an alteration of the fibrinogen, we have also been led to the opinion that these data cannot be explained on the basis of two separate components of prothrombin. We have shown (13) that after treating plasma with aluminum hydroxide to give an A factor plasma, it is still possible to elute a complete prothrombin from the aluminum hydroxide. Obviously, if the A and B factors were distinct separate components of prothrombin, the eluate should contain only component B, since component A is still demonstrable in the adsorbed plasma. Further, our demonstration that the

A factor is present in serum, free of both fibrinogen and thrombin, again indicates that the A factor is not a constituent of prothrombin.

Since it has been shown that the observed data cannot be explained on the basis of either an altered fibrinogen or two separate components of prothrombin, we wish to suggest a third explanation of these data. We suggest that prothrombin be considered as a unitary principle as claimed by Loomis and Seegers (8). The prothrombin molecule, however, has as part of its structure a labile group which is inactivated by various procedures, such as storage both at neutral pH and more rapidly at more alkaline pH. This inactivated group can be partially reactivated by some other protein or proteins present in both plasma and serum.

Prothrombin B should, therefore, be considered as prothrombin which has become partially inactivated by an alteration of the labile group of the molecule.

Prothrombin A must, for the present, remain as an unknown factor of which little is known except that it is not fibrinogen, as claimed by Loomis and Seegers (8). Their reactivation of inactivated prothrombin by the addition of fibrinogen preparations can only be interpreted as the result of the presence of traces of the reactivating factor as a contaminant of their fibrinogen. This interpretation is confirmed by the fact that Deutsch and Gerarde (14), using fibrinogen prepared by the method described by Seegers et al. (15), failed to find any effect on the prothrombin time of stored plasma. We do not consider that it is advisable to attempt to formulate a name for this factor at the present time.

Studies are in progress in this laboratory regarding the activity of various fractions of plasma obtained by electrophoretic fractionation. These indicate that the reactivating factor is present in most of the recognized electrophoretic fractions of plasma. These data will be published shortly.

SUMMARY

1. The restoration of prothrombin activity to stored plasma, and similar preparations, by aluminum hydroxide plasma or dicumarol plasma cannot be explained on the basis of a change in the reactivity of fibrinogen.

2. It is suggested that this inactivation of prothrombin is the result of inactivation of a labile group in the prothrombin molecule. This group can be reactivated by other proteins present in plasma and serum.

We wish to acknowledge the technical assistance of Miss Annabel Avery.

ADDENDUM

Since this paper was sent to press, an extensive study by Paul A. Owren ("The Coagulation of Blood. Investigations on a New Clotting Factor", Thesis, J. Chr. Gundersen, Oslo, 1947) has come to our attention. He has concluded that pure prothrombin cannot be converted to thrombin in the presence of thromboplastin and calcium unless a further factor, which he has designated as factor V, is present in the mixture. The properties of factor V are in many ways similar to those of the activator, factor A, discussed in this paper. Studies are in progress to determine whether factor V and factor A are identical.

Ware, Guest and Seegers (J. Biol. Chem. **169:** 231, 1947) have also reported on a factor in plasma which accelerates the activation of prothrombin by thromboplastin and calcium. Further investigation is necessary in order to demonstrate the identity or non-identity of these three activators.

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DISAPPEARANCE OF UROPEPSIN FROM THE URINE OF GASTRECTOMIZED CATS[1]

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Uropepsin is a proteolytic enzyme resembling gastric pepsinogen which occurs in the urine of normal animals and man. Following complete gastrectomy, or removal of the gastric mucosa, in dogs, it has been reported by several investigators that uropepsin can no longer be detected in the urine by means of the fibrin method (Frouin and Delezenne, 1; Matthes, 2; Wolgemuth, 3; Calar, 4). The fibrin method has long been abandoned as unreliable for uropepsin, yet the application of more acceptable methods to the confirmation of these observations has not been made. In re-investigating the physiology of uropepsin, the initial problem was to confirm the gastric source of this enzyme using a better substrate.

In selecting the cat as the best animal on which to study the physiology of uropepsin, we have been guided by the observations of Peczenik and Kawahara (5) who report that of the common laboratory animals the cat eliminates uropepsin in the highest concentration and has the highest "anti-pepsin" titer in its blood. We have found that gastrectomy in the cat is followed in 2 to 6 days by a complete disappearance of uropepsin from the urine when assayed by a hemoglobin method for pepsin adapted for use on the urine.

METHOD. Four cats were prepared for this study by being dewormed (tetrachlorethylene) and then conditioned to a standard diet and routine. Each cat was housed in a cage supplied with a wire false-bottom, so that fecal-free urine could be collected from the sloping floor into a 250 ml. beaker containing 5 ml. of 0.5 N hydrochloric acid and some mineral oil. The diet consisted of frozen chopped horse meat, one pound of which was reground and cooked daily. Each cat received 50 grams of the meat stew and 25 ml. of fresh milk three times a day. The cages were scrubbed daily. The urine was collected once a day and stored in the refrigerator until analyzed, usually the same day.

The operations were performed under ether anesthesia on animals that had been fasted 24 hours and atropinized 30 minutes previously (1 mgm. atropine sulfate subcutaneously). The technique of the surgery was the same as used for dogs, and described by Ivy, Morgan and Farrell (6). The animals were allowed water on the second post-operative day and offered food (fresh boiled milk or meat broth) on the third. If food and water were refused, physiological saline (50 ml.) was administered subcutaneously. The appetite of all animals

[1] Aided by a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

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was depressed following the operation and a wide variety of food was offered to induce them to eat. Most success was achieved with sardines, and hard boiled yolk of egg.

The analysis consisted in volume measurements and uropepsin assays. Uropepsin was assayed by the hemoglobin method as adapted for urine in a previous study (Bucher, 7). All cats micturated as they awakened from the ether. The post-operative urines are therefore true post-operative excretions. The pH was taken on the urine and on sample digests of the urines to insure that the samples had been preserved in the acid state and that the digestions were conducted within the optimal range of pH 2.0 to 3.3.

TABLE 1

Summary of the preoperative and postoperative uropepsin output

CONTROL STUDIES BEFORE GASTRECTOMY	CAT 1	CAT 2	CAT 3	CAT 4
Period of observation (days)	13	16	18	23
Mean 24-hr. urine (ml.)	91.6	78.8	98.4	93.0
Mean 24-hr. uropepsin output*	31.9	44.5	41.8	38.7
Body weight (kilo)	2.85	3.15	2.20	2.10
OBSERVATIONS FOLLOWING GASTRECTOMY				
Uropepsin in first voiding*	19.8	Lost	11.3	18.4
Uropepsin in second voiding*	3.7	3.7	Died	None
Uropepsin in third voiding*	None	None		5.6
Subsequent collections	None	None		Trace†
Period of observation (days)	14	27	1	117
Days till zero uropepsin	2	3		7
Total postoperative uropepsin output	23.5	3.7	11.3	42.6
Mean 24-hr. urine (ml.)	37.0	48.0	44.0	45.4
Body weight at end of record (kilo)	2.15	2.05	2.10	2.09
Status of animal at end of record	Dead	Living	Dead	Living
Survival period	Lived 14 days	Lived 64 days	Lived 1 day	Lived 144 days

* Uropepsin is expressed here as milligrams of tyrosine.

† On the 5th, 7th, and 14th postoperative collections a trace of uropepsin was present. Assays made at the 88th and 117th days, when the animal had regained its preoperative weight, and was eating well, showed no uropepsin was present.

Results and discussion. It may be seen from table 1 that gastrectomy in cat 1 resulted in the complete absence of uropepsin from the urine after the second voiding on the second post-operative day. In 8 additional specimens in the 14-day survival period there was no uropepsin. In cat 2, likewise, uropepsin disappeared entirely by the second voiding on the third post-operative day. In all of the 18 subsequent urine specimens, no uropepsin was ever found. The appetite of this animal was extremely poor throughout the survival period of 64 days and the weight loss was never arrested. Cat 3 lived but one day. The most interesting data were obtained from cat 4. This animal did not micturate until the 5th post-operative day. The second urine on the 7th day con-

tained no uropepsin, yet intermittently a trace of enzyme activity was detected, i.e., on the 8th, 11th, 13th, 21st and 88th post-operative days. At two months post-operatively, this animal was shipped to Philadelphia. Upon arrival, the body weight was 1.50 kilo as compared to the pre-operative weight of 2.10 kilo, and the hemoglobin of 8.3 grams showed that a definite anemia was present. (Normal hemoglobin for cats is 11 to 12 grams per cent according to data compiled by Wintrobe (8); the mean value for 3 normal cats in our laboratory was 11.9 grams.) The appetite was so poor that cream fortified with B vitamin complex was force fed with a medicine dropper and liver extract was given parenterally. During the next two months the appetite improved, the body weight increased to 1.93 kilos and the animal became playful. A freshly voided urine specimen *showed no uropepsin*. Thus in all three of the cats which survived, uropepsin elimination ceased within 2 to 6 days following gastrectomy, and in the one animal which attained a satisfactory nutritional status in the 3rd to 5th post-operative month, there was no evidence of a return of uropepsin elimination.

These results are consistent with the view that uropepsin arises at the stomach, is carried in the blood and eliminated in the urine. The removal of the stomach removed the tissue source and in a few days, when the kidneys had cleared the enzyme from the blood, uropepsin elimination fell to zero.

The objection might be made that the failure to eliminate uropepsin was the result of the poor nutritional state of the animals. While a reduced food intake can significantly depress uropepsin output, under the conditions of simple starvation, uropepsin elimination is never completely abolished. In our experience, the refusal of food by normal cats is always followed by a marked reduction in uropepsin output, but it never falls to zero. In the gastrectomized cats, the uropepsin output fell to zero about the time they began again to eat, following the operation. The complete absence of uropepsin in the freshly voided urine of cat 4 at a time when this animal was eating well and gaining weight is further evidence that the absence of uropepsin was due to the loss of the tissue of origin and not to inadequacies of food intake. The "trace" amounts of uropepsin assayed in this cat during the first post-operative months may have been due to the presence of gastric tissue at the anastomosis, for upon the death of this animal a few patches of gland resembling that of the cardiac stomach were found in histological sections of the esophageal-duodenal anastomosis.

As a matter of history, the first really effective removal of the stomach of an animal was achieved in 1894 by Carvallo and Parchon (9) on a cat which lived 6 months. We believe this study to be the second recorded observation on gastrectomized cats. In view of the well known relation between gastrectomy and anemia in other animals, and the severely reduced uropepsin elimination observed in patients with pernicious anemia (Rothschild (10), Farnsworth, Speer and Alt (11)) the progressive anemia observed in cat 4 is noteworthy.

The anemia was discovered the 10th week after the gastrectomy. During the next 10 weeks, when the animal was gaining weight (1.50 to 2.09 kilo) the hemoglobin fell steadily from 8.3 to 7.5 and finally to 6.2 grams per cent. In this period, liver extract had been injected twice (15 units at a time) primarily

to stimulate the appetite. In the 19th post-operative week, the blood of this cat was studied, along with that of 3 normal cats to determine whether the anemia was of the secondary type which develops as a consequence of gastrectomy in other animals, i.e., the rat (12), the dog (6), the hog (13), the monkey (14) and man (15). The erythrocyte counts, hematocrits and the hemoglobin values, as well as the derived figures for mean corpuscular hemoglobin (M.C.H.), mean corpuscular volume (M.C.V.), mean corpuscular hemoglobin concentration (M.C.H.C.) and the common blood indices for color, volume and saturation are listed in table 2. In comparison to the normal cats in the same laboratory (evaluated with the same instruments) the anemia of the gastrectomized cat was of the hyperchromic, macrocytic type; by comparison to the normal values recorded in the literature (8), it was of the hyperchromic normocytic type-

TABLE 2

Blood data from normal and gastrectomized cats

	NORMAL VALUES WINTROBE (8) APPENDIX A	AVERAGES ON 3 NORMAL CATS	CAT 4, 19 WEEKS WITHOUT A STOMACH	CAT 4 AT DEATH 3 DAYS AFTER 1 DOSE IRON
R.B.C. (million/cmm.)	7.84	7.55	3.68	4.96
Hematocrite* (cc./100 cc.)	40.0	37.9	21.7	27.0
Hemoglobin† (gm./100 cc.)	11.6	11.9	6.2	7.4
M.C.H. (micromicrograms)	15.0	15.7	17.2	14.9
M.C.V. (cubic microns)	57.0	50.2	59.2	54.4
M.C.H.C. (per cent)	27.0	31.3	28.5	27.4
Color Index	1.0	1.05	1.13	1.00
Volume Index	1.0	0.97	1.15	1.07
Saturation Index	1.0	1.08	1.00	0.93
R.B.C. diameter (micron)	5.9		6.2	

* Wintrobe tubes.

† The oxyhemoglobin measured with Klett-Summerson photoelectric colorimeter

There was no poikilocytosis. The anemia customarily observed in gastrectomized animals is of the hypochromic and either microcytic or normocytic type which responds well in some species to parenterally administered iron.

We had hoped to follow the response to injected iron, but unfortunately the cat developed a generalized infection and died 3 days after 0.75 mgm. ferric ammonium citrate was administered. The findings on the heart blood taken at the moment of death are given in table 2, but considering the toxic state of the animal, no significance can be attached to them. There can be no doubt that, prior to the attempted iron therapy in our gastrectomized cat, a non-regenerative anemia (oligocythemia) developed which was neither hypochromic nor microcytic.

CONCLUSIONS

1. In three cats which survived total gastrectomy 14, 64 and 144 days respectively, uropepsin disappeared completely from the urine in 2 to 6 days following the operation.

2. There was no evidence of a return of uropepsin elimination in one cat which survived 20 weeks, had regained the pre-operative weight and was eating well.

3. The cat which survived 20 weeks developed a severe anemia which was neither of the hypochromic nor microcytic type.

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THE ALLEGED EXCRETION OF HISTAMINE IN PARATHYROIDECTOMIZED DOGS

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It has been reported by W. F. Koch (1913) that three out of six parathyroidectomized dogs under his observation excreted in their urine a considerable amount of a substance which by its melting point, chemical analysis and positive diazo reaction was recognized as histamine. He also found that all the operated animals excreted a large amount of methyl guanidine. Since the publication of Koch's paper, Bryan, Minot and Chastain (1933) have shown that the appearance of guanidine compounds is not a direct result of parathyroidectomy but of the accompanying dehydration. As regards the alleged excretion of histamine, no further investigations have been reported.

The amount of histamine base, excreted in the course of the 5 to 7 days of survival of the animals, was in one of Koch's dogs 0.34 and in another 0.24 gram. The total amount of urine excreted by one of the animals during the period of survival was 2250 cc. which gives an average concentration of histamine base of 100 to 150 μg/cc. The maximal concentration of histamine base ever observed in dog's urine on a heavy meat diet was about 30 μg/cc. and after administration of conjugated histamine, 50 μg/cc. (Anrep, Ayady, Barsoum, Smith and Talaat, 1944); the result of Koch's observations obviously required reinvestigation, especially since the author provides no analysis of normal dog's urine and since, at the time of his experiments, it was not known that normal urine contains histamine.

Our investigation was made on 7 parathyroidectomized female dogs weighing 9 to 14 kilos. As much thyroid tissue as possible was left intact in three dogs; in the other four, the thyroid was removed together with the parathyroids. One dog of the first group and one of the second survived the operation, probably on account of some parathyroid tissue being overlooked. The other five dogs developed the usual symptoms of parathyroid deficiency and died within 5 to 8 days after the operation. The urine was collected by catheter at 6 to 8 hour intervals. Long before the observations were begun all the dogs had a longitudinal incision made in the perineum to expose the urethra so as to facilitate the introduction of the catheter; 24 hour samples of the urine were analysed every day, beginning from about 10 days before the parathyroidectomy. Chloroform was added to the urine to prevent putrefaction. The urine extracts, for the determination of the total and free histamine, were prepared as described by Anrep and co-workers (1944) and the assays were made on the guinea-pig's ileum by the method of Barsoum and Gaddum (1935). The dogs were kept on a mixed diet consisting of white cheese, boiled rice, milk and one egg per day. Some of the animals were given 100 to 250 grams' meat instead of

cheese. After the operation the dogs consumed only a part of the food and later refused it altogether.

RESULTS. The two dogs which survived the operation showed no change in their histamine excretion. One of them receiving meat, excreted 28 mgm. histamine (calculated as diphosphate) during the 10 days preceding the operation and 30.5 mgm. during the same period after the operation. The second dog received no meat; it excreted an equivalent of 1.8 and 1.5 mgm. histamine diphosphate during the 10 days before and after the operation respectively. The results obtained are summarised in the table in which the total amount of histamine and of the urine excreted during the survival time and during the same number of days immediately before the operation are compared.

TABLE 1

Histamine excretion before and after parathyroidectomy

The total amount of histamine (calculated as diphosphate) in milligrams excreted during the period of survival is compared with the excretion which took place during the same number of days immediately preceding the operation.

	WEIGHT IN KILOS		DAYS OF SURVIVAL	HISTAMINE IN MGM. (AS DIPHOSPHATE)		URINE IN LITRES	
	Before operation	At death		Before	After	Before	After
1	13.1	12.9*	survived	28.0	30.5	4.61	6.39
2	10.6	11.2*	survived	1.8	1.5	2.95	2.49
3	9.5	7.2	8	7.7	5.1	2.70	1.83
4	12.6	8.5	8	1.9	1.1	3.97	2.17
5	12.7	9.6	6	6.9	7.4	2.60	2.14
6	13.2	10.6	7	1.4	1.7	3.03	1.04
7	14.5	12.5	5	14.5	10.1	3.90	2.60

* Weight 10 days after the operation.

Almost all the histamine was excreted in a conjugated form.

It can be seen from the table that in all the operated dogs the excretion of histamine was not greatly changed. It is likely that the very large amount of histamine detected by Koch might have been due to accidental causes, such as a bacterial formation of histamine after the urine had been collected or a contact with the feces during its collection in the metabolic cage. Koch collected the entire urine excreted during the survival period and analysed it in one lot. In our work the urine was analysed in 24 hour samples.

The daily excretion of histamine showed in some animals considerable variation before and after the operation. It always diminished towards the end of the survival period. In four animals the histamine excretion began to diminish two to three days after the operation. The diminution continued together with the decline of urine formation and with the general deterioration of the animal. The concentration of histamine per cubic centimeter of urine showed, in these dogs, no significant change until the last few days of survival when it gradually declined. In one dog the histamine excretion was maintained at the

pre-operative level up to the last day when it suddenly dropped almost to zero. Since the diminution of the urine flow in this dog, as in all the others, was progressive, the concentration of histamine per cubic centimeter of urine became conspicuously increased especially to the end. On the last day of survival the concentration of histamine like its total excretion abruptly declined.

It has been shown by Anrep and co-workers that all animals excrete, in addition to conjugated histamine, traces of free histamine. In normal dogs free histamine occurs in concentrations of 0.01–0.3 μg per cc. of urine. The urine of parathyroidectomized animals did not differ in this respect from normal urine.

SUMMARY

The observations of W. F. Koch that large amounts of histamine can be detected in the urine of parathyroidectomized dogs could not be confirmed. After the operation, histamine excretion either gradually declines until the death of the animal or is maintained at the pre-operative level and rapidly drops on the last day of the animal's survival.

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EFFECT OF ADRENAL CORTEX EXTRACT UPON THE TOLERANCE OF THE EVISCERATED RAT FOR INTRAVENOUSLY ADMINISTERED GLUCOSE

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In these experiments eviscerated rats were given continuous intravenous infusions of glucose with and without adrenal cortex extract and insulin for periods of 2, 4 and 24 hours. During the first 4 hours there was no effect of adrenal cortex extract upon the glucose level of the blood. Within a 24-hour period adrenal cortex extract definitely depressed the tolerance for glucose in those rats which received insulin. In the absence of insulin the effect of adrenal cortex extract upon glucose tolerance was either absent or questionable.

METHODS. Male rats of the Sprague-Dawley strain were used. The diet was Purina Dog Chow. When the rats reached a weight of 185 to 205 grams, the inferior vena cava was ligated between the liver and kidneys in order to cause the development of a collateral circulation. Asepsis was preserved in this operation. When the animals reached a weight of 250 (±2) grams they were anesthetized (intraperitoneal injection of 18 mgm. of cyclopentenyl-allyl-barbituric acid sodium) and eviscerated by the procedure described by Ingle and Griffith (1). All of the intra-abdominal organs were removed except the adrenals and the kidneys.

The adrenal cortex extract was prepared from beef adrenal glands and represented 40 grams of whole gland per cubic centimeter. It was free from alcohol. Intravenous injections of solutions containing varying concentrations of glucose and 0.9 per cent sodium chloride with and without crystalline zinc insulin (Lilly) (4 units per 24 hrs. per rat) and adrenal cortex extract (Upjohn) were made by two continuous injection machines which delivered fluid from each syringe at the rate of 20 cc. in 24 hours. Syringes of the Luer-Lok type were selected to deliver 20 cc. with a stroke of 65 mm. Two syringes were operated by one machine and six syringes by the second machine. Each machine was powered by a synchronous motor, and the reduction of motion was achieved by a precision-built system of gears so that an exact control of the rate of injection was maintained.

The infusions were made into the saphenous vein of the right hind leg and were started within five minutes following the removal of the liver. The animals were secured in a supine position on an animal board. In these liverless animals one initial dose of the barbiturate was sufficient to maintain effective anesthesia throughout the experiment. The temperature of the room was maintained at 75 to 78 degrees F. and the humidity at 30 to 35 per cent of saturation. The glucose load is expressed as milligrams of glucose per 100 grams of rat per hour (mgm./100/hr.).

The analyses of blood glucose were made by the method of Miller and Van Slyke (2) and of urine glucose by the method of Benedict (3). This method for the determination of blood glucose measures small amounts of non-fermentable reducing substances which accumulate in the blood of eviscerated rats. Samples of blood (0.1 cc.) were taken from the tail just prior to the beginning of the infusions, after 2 and 4 hours of infusion in experiment 1 and from the jugular vein at the end of 24 hours of infusion in experiment 2. The excreted urine was collected and preserved with thymol. The urine remaining in the bladder was added at necropsy.

Experiments and results. In experiment 1 (fig. 1) 22 pairs of eviscerated rats were infused with glucose (18/100/hr.) without insulin and 21 pairs were infused with glucose (72/100/hr.) plus insulin for periods of 2 and 4 hours. One

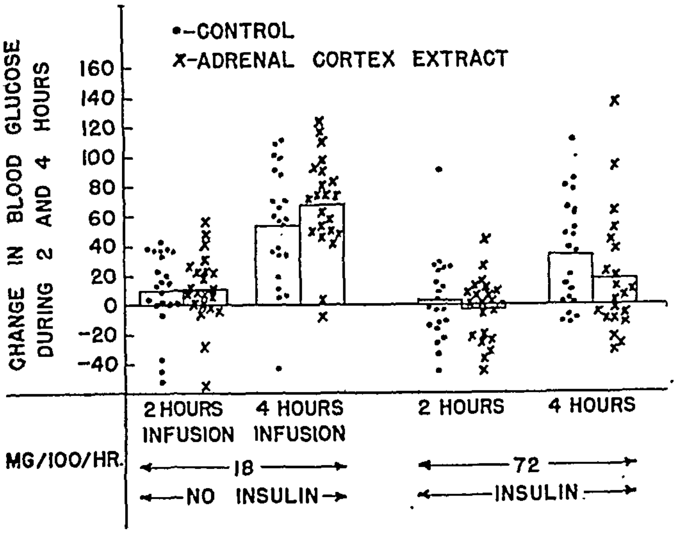

Fig. 1. Tolerance for glucose administered to eviscerated rats with and without insulin and adrenal cortex extract. Two and four hours of continuous intravenous injection. Means and individual values.

rat of each pair received adrenal cortex extract. There was no significant effect of adrenal cortex extract upon the level of blood glucose within 4 hours of infusion. In each group there was an average rise in blood glucose during the second two hours of infusion as compared to the first two hours of infusion.

In experiment 2 (fig. 2) the tolerance to glucose was determined over a 24-hour period. A series of eviscerated rats without insulin were studied at glucose loads of 4, 6, 8 and 10 mgm./100/hr. Twenty to 22 pairs of rats were tested at each of the four levels. A series of similar animals were given insulin with glucose loads of 30, 32, 34, 36, 38 and 40 mgm./100/hr. Eleven to 12 pairs of rats were tested at each of the six levels. One rat of each pair received adrenal cortex extract. Among the animals which were not given insulin the effect of adrenal cortex extract upon the tolerance of these animals for glucose was either absent or questionable (8/100/hr. level, fig. 2). Among the animals given insulin the administration of adrenal cortex extract caused a significantly greater rise

in blood glucose than was shown by the control animals at each level of glucose load.

At the higher loads of glucose some of the insulin-treated rats showed glycosuria. At a load of 36/100/hr. 10 of the 12 rats given adrenal cortex extract excreted from 24 to 200 mgm., and one of 12 control animals excreted 34 mgm. of glucose. At a load of 38/100/hr., 2 of the 11 rats given adrenal cortex extract excreted 36 and 111 mgm., and none of the 11 control animals had glycosuria. At a load of 40/100/hr., 9 of 11 rats given adrenal cortex extract excreted 26 to 103 mgm. of glucose, and 4 of 11 control rats excreted 26 to 46 mgm.

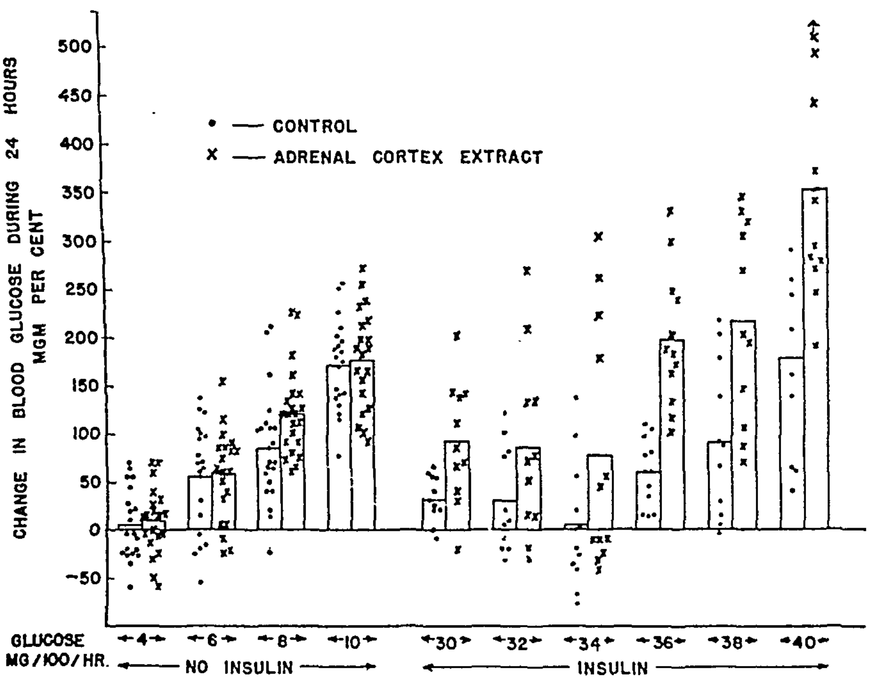

Fig. 2. Tolerance for glucose administered to eviscerated rats with and without insulin and adrenal cortex extract. Twenty-four hours of continuous intravenous injection. Means and individual values.

Similarly, some of the animals given glucose without insulin had glycosuria. At a load of 8/100/hr., 4 of 22 rats given adrenal cortex extract excreted 32 to 118 mgm.; and 3 of the 22 control rats excreted 28 to 150 mgm. At a load of 10/100/hr., 11 of 20 rats given adrenal cortex extract excreted 39 to 109 mgm., and 8 of the 20 control rats excreted 26 to 110 mgm.

Discussion. There have been a number of earlier observations which indicate that the adrenal cortical hormones affect metabolic processes in the absence of the liver. In studies (4) on the eviscerated, nephrectomized, adrenalectomized rat given glucose without insulin it was shown that adrenal cortex extract had a favorable effect upon survival times and upon ability to work. Russell (5) found that rats which had been adrenalectomized several days prior to "functional evisceration" had a higher rate of glucose utilization than non-adrenalectomized, eviscerated rats. This change due to adrenalectomy could be prevented by the

administration of adrenal cortex extract at the time of evisceration or by the administration of 11-desoxycorticosterone prior to evisceration. Adrenal cortex extract, but not 11-desoxycorticosterone acetate, prevented the rise in glucose utilization in the hypophysectomized, eviscerated rat. It was suggested by Russell that the apparent effect of adrenal cortical insufficiency upon glucose utilization in the eviscerated rat may have been due to the development of shock in these animals and that the effect of the cortical hormones was to prevent shock. Similarly, it was shown by Roberts (6) that adrenalectomy at the time of evisceration of the fasted rat produced a 50 per cent reduction in survival time and an increased rate of blood sugar disappearance. Treatment with an oil concentrate of hog adrenal gland had a small moderating effect in the adrenalectomized, eviscerated rat. Roberts has suggested that the primary effect of adrenalectomy in the eviscerated rat may be hemodynamic rather than one of increased glucose utilization. Selye and Dosne (7) and Reinecke (8) have reported that adrenal cortex extract was without effect upon the blood glucose level of the liverless rat. The amounts tested were small, and it was not used in the insulin-treated animal.

The data of the present study confirm the tentative conclusions of an earlier report (9) from this laboratory. Does the effect of adrenal extract in inhibiting the utilization of glucose by the eviscerated rat represent the mechanism which causes adrenal diabetes and insulin resistance in the intact rat (10)? The factor of hepatic gluconeogenesis is operative in the intact animal, but it is probable that inhibition of carbohydrate utilization (oxidation, storage and conversion) is the major factor. It seems reasonable to postulate that the principal mechanism causing adrenal diabetes in the intact rat is extra-hepatic and is identical with the mechanism whereby the utilization of glucose is inhibited in the eviscerated rat. Proof for this hypothesis is lacking. It would also be possible to explain the data of the present report by assuming that the cortical hormones stimulated gluconeogenesis in the kidney. These studies should be repeated in the eviscerated, nephrectomized rat.

The changes in tolerance for carbohydrate in these experiments are small in comparison with the changes in tolerance for carbohydrate which represent adrenal diabetes in the intact rat. As a possible explanation, the duration of the present studies was 24 hours, whereas several days are required for the full development of adrenal diabetes (10). Moreover, the intact rat (11) can utilize much greater amounts of carbohydrate than the eviscerated rat.

These results may relate to the findings of Price *et al.* (12) that certain adrenal principles modify the hexokinase reaction by intensifying the inhibitory effect of anterior pituitary extract upon the action of insulin. Is the effect of adrenal cortical hormones upon the peripheral utilization of carbohydrate dependent upon the presence of insulin? Our results up to the present time would support this conclusion. However, we have not studied the effect of adrenal cortical insufficiency upon glucose utilization in the eviscerated rat in the presence and absence of insulin. Both Russell (5) and Roberts (6) have reported that adrenalectomy accelerated the fall of blood glucose in eviscerated rats which received no insulin.

The favorable effect of adrenal cortex extract upon the survival time and work performance of adrenalectomized, nephrectomized, eviscerated rats has been noted (4). How does this effect of the cortical hormone relate to its inhibitory effect upon carbohydrate utilization as observed in the present study? Further investigation of the effect of the cortical hormones upon work performance and collateral changes in glucose utilization in the adrenalectomized, eviscerated rat should throw light on the rôle of the cortical hormones in body economy.

SUMMARY

Male rats (185–205 grams) of the Sprague-Dawley strain were caused to develop a collateral circulation by ligation of the inferior vena cava. At a weight of 250 ± 2 grams the animals were anesthetized (cyclopal), and all of the intra-abdominal organs were removed except the kidneys and adrenals. Infusions into the saphenous vein were made by continuous injection machines which delivered fluid at a rate of 20 cc. in 24 hours per rat. The change in the level of blood glucose during the subsequent 24 hours was the index of glucose tolerance.

In experiment 1, 22 pairs of eviscerated rats were infused with glucose (18/100/hr.) without insulin, and 21 pairs were infused with glucose (72/100/hr.) plus insulin for periods of 2 and 4 hours. The administration of adrenal cortex extract in large amounts to one rat of each pair had no significant effect upon the level of blood glucose within 4 hours of infusion.

In experiment 2 the tolerance to glucose was determined over a 24-hour period. Eviscerated rats without insulin were given glucose loads of 4, 6, 8 and 10/100/hr. Twenty to 22 pairs of rats were tested at each of the four levels. Similar animals were given insulin with glucose loads of 30, 32, 34, 38 and 40/100/hr. Eleven to 12 pairs of rats were tested at each of the six levels. The administration of adrenal cortex extract to one rat of each pair given insulin caused a significant rise in blood glucose above that of the control animals but had only a questionable effect in the animals not given insulin.

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COMPARATIVE EFFECTIVENESS OF ALBUMIN, GLOBIN, HEMOGLOBIN, GELATIN, OXYPOLYGELATIN, SALINE, RINGER'S, BLOOD AND PLASMA UPON THE SURVIVAL OF RATS SUBJECTED TO STANDARDIZED SCALD BURNS[1]

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A survey of the literature on the treatment of burn shock discloses disagreement among the many investigators regarding the therapeutic efficacy of the various infusion fluids. It appears that, in general, emphasis is shifting from the concept of plasma deficiency and replacement to one of total body fluid distribution as influenced by deficiencies not only in plasma colloid, but also in the extracellular electrolytes, particularly sodium.

The criteria used by the various investigators for judging the comparative efficacy of whole blood, red cell suspensions, plasma, saline, balanced ion solutions and plasma substitutes have been different. The species of experimental animals, the conditions of the animals and the conditions under which the experiments have been carried out have differed greatly. Hence, the lack of agreement concerning the comparative efficacy of the infusion fluids tested is not surprising.

In the experiments to be discussed below, the major criterion of long-term survival (ten day period) has been used to judge the relative efficacy of the various fluids studied. Hematocrit data have also been obtained and have demonstrated that although some fluids may adequately control the hemoconcentration following thermal injury they do not necessarily favorably affect survival.

The group of infusion fluids listed in table 1 were tested for their ability to promote the survival of rats subjected to a standardized burn of approximately 50 per cent lethality. A cursory examination of this table suggests that rats sustaining this degree of thermal injury were not in critical need of colloid replacement since the use of a physiological saline solution (2 per cent of body weight) was followed by survival rates not significantly different from those obtained with whole blood, plasma, human serum albumin or a gelatin solution in like amounts.

Modifications in the experimental procedure were made later in order to obtain an injury of 100 per cent lethality in untreated animals. When such an injury was obtained, it was found that physiological saline solution did not allow the same survival rate as a solution of serum albumin in saline in volumes of

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4 per cent of body weight. However, when a volume of 10 per cent of the body weight of physiological saline solution was infused no difference in survival rate was obtained between the albumin solution and physiological saline solution (table 3).

In order to test further the efficacy of sodium chloride a third series of experiments was performed in which two concentrations of sodium chloride solutions were compared with the following: plasma, serum albumin (salt rich and salt poor), balanced ion solutions with and without potassium and a Ringer-Locke solution.

TABLE 1

Postburn survival rates of rats receiving 90°C. burn for 15 seconds

2% body weight infused—1 infusion

INFUSION FLUID	NO. OF RATS	PER CENT SURVIVAL POSTINFUSION					
		12 hours	24 hours	48 hours	72 hours	5 days	10 days
Whole blood	20	100	100	95	95	95	95
Plasma	20	95	95	95	95	95	95
Human serum albumin Cohn—25 per cent	20	95	95	95	95	95	95
Saline 0.85 per cent	20	100	96	90	90	90	90
Albumin 5.0 per cent	20	100	90	90	90	90	90
Knox P-20 gelatin 4 per cent solution	20	100	100	100	90	90	85
Red blood cells in 0.85 per cent saline	20	95	90	90	90	85	85
Oxypolygelatin 37°C. 5 per cent solution Pauling and Campbell	20	90	80	75	75	75	75
Human hemoglobin 7 per cent solution Sharpe and Dohme	20	85	75	75	75	75	75
Oxypolygelatin 22°C. 5 per cent solution	20	80	60	60	55	55	55
Controls	56	89	59	55	47	47	47
Rat globin 1.5 per cent—Strumia	8	75	63	50	38	38	38

Results of these experiments have led us to conclude that under the conditions of our experiments survival rates can be correlated with the sodium chloride concentration and the fluid volume replacement.

EXPERIMENTAL. In all experiments Wistar stock rats weighing between 190 and 210 grams were employed. Rats weighing between 150 and 190 grams obtained from the Wistar Institute were maintained in our laboratory on a diet of Purina Dog Checkers supplemented with whole milk for at least one week prior to use. Healthy animals regularly gaining weight were selected from the stock room and moved into the experimental room on the day preceding an experiment. On the morning of the experiment the rats were again carefully examined and weighed. Equal numbers of healthy rats weighing between 190 and 200 grams, and 200 and 210 grams were selected. These by random selection were divided into experimental groups each containing equal numbers of the two weight ranges.

The unshaved rats under ether anesthesia were subjected to a standardized back burn (1) involving 32 ± 2 per cent of the total body surface in water at 90°C. ± 0.25°C. In order to minimize variation all the burning was done by the same individual.

In the first series of experiments (table 1) an approximate 50 per cent lethality in the untreated animals was obtained by immersion for fifteen seconds. In the other experimental groups all animals were immersed for thirty-five seconds which produced a burn that was 100 per cent lethal to the untreated animals.

Burned areas of the rats were calculated from planimetric measurements of the injured areas made at death or sacrifice of the animals (1).

All infusions were made under aseptic conditions without anesthesia into the internal saphenous vein exposed by a small skin incision. Bleeding following infusion was controlled by applying pressure at the site of the needle puncture with a sterile gauze compress.

Hematocrit samples were obtained by nicking the lateral tail vein and drawing up approximately 0.01 cc. of blood into uniform bore capillary tubes wet with heparin solution. One end of the capillary tube was then sealed in a flame from a microburner. All samples were centrifuged at 2,500 r.p.m. for thirty minutes (radius 14 cm. to bottom of tube). Equal numbers of hematocrit samples were obtained in simultaneous experimental groups, as the handling of the animals during this procedure was previously found by one of us (1) to influence the survival rate.

Whole blood for infusion was prepared on the day preceding its use approximately eighteen hours before it was infused. Large rats, 300 to 400 grams, were fasted for twenty-four hours. They were then lightly anesthetized with ether and injected intravenously with 100 Toronto units of Connaught heparin solution. The blood was withdrawn from the left ventricle into a sterile syringe wet with heparin solution. This blood was stored in sterile tubes at approximately 7°C. until just prior to its use.

The plasma was prepared from whole blood obtained in the manner just described. After withdrawal of the whole blood it was centrifuged in sterile 15 cc. graduated centrifuge tubes packed in cracked ice at 2,500 r.p.m. for thirty minutes (radius 20 cm. to bottom of tube). The plasma was withdrawn into cold sterile 10 cc. syringes fitted with 3 inch 18 gauge hypodermic needles. Care was exercised during the removal of the plasma in order to avoid contamination by the components making up the buffy coat. Plasma prepared from six animals at a time was pooled and stored in sterile containers at 7°C. for approximately eighteen hours prior to infusion.

Red blood cells, to be suspended in physiological saline solution for infusion, were obtained from whole blood collected in the manner described above the day preceding their use. On the day of the experiment the whole blood was centrifuged in tubes packed in cracked ice for thirty minutes at 2,500 r.p.m. (radius 20 cm. to bottom of tube). Following centrifugation, the plasma, the components making up the buffy coat, and the top layer of red cells were removed. To the remaining volume of red cells was then added an equivalent volume of physio-

logical saline solution. This cellular suspension was gently agitated before each infusion in order to obtain homogeneity of the mixture.

Since seasonal variations in the animals and external conditions, such as room temperature and humidity, affected the survival rates, only those infusion groups in experiments which were done at the same season of the year and under comparable environmental conditions have been compared.

RESULTS. *Efficacy of fluid replacement (2 per cent of the body weight) in burns of 50 per cent lethality.* In the series of experiments listed in table 1 all animals received a fifteen second burn as described above. Two hours following the burn infusions of the various infusion fluids amounting to 2 per cent of the body weight, except the 25 per cent albumin solution, were introduced. The concentrated albumin solution was given in one fifth of this volume, containing an amount of albumin equivalent to 2 per cent of the body weight of the 5 per cent solution.

In these experiments two infusion fluids were tested simultaneously in equal numbers of animals. Untreated controls were recorded at various intervals up to ten days.

With respect to the degree of injury studied under the standard conditions outlined above no significant difference in efficacy existed between the following infusion fluids: whole blood, plasma, 0.85 per cent physiological saline solution, 5 per cent human albumin solution, the same dose of albumin in 25 per cent solution, Knox P-20 gelatin (4 per cent) and erythrocytes in 0.85 per cent saline. All of these infusion fluids significantly improved the survival rates of the injured animals compared to the untreated control animals as indicated by a P value of 0.02 or less calculated from the formula of Fisher for fourfold tables (2).

Oxypolygelatin heated to 37°C. and human hemoglobin significantly increased survival. However, these two infusion fluids were less effective than those discussed above in increasing survival as indicated by a probability value of 0.05.

Oxypolygelatin when administered at 22°C. did not significantly raise the survival rate as compared to the untreated controls.

Modified rat globin received through the co-operation of Dr. Max Strumia resulted in a slightly lower survival rate than was observed in the controls. This material was an experimental batch and was not regarded as an entirely satisfactory product on the basis of the chemical criteria set up for controlling the production of modified human globin. Modified human globin was not well tolerated by the rat.

Table 2 gives the average hematocrit values as percentages of the pre-burn values. A pre-infusion value taken just prior to infusion or two hours postburn and four postinfusion values taken at thirty minutes, two hours, five hours, and ten hours respectively were recorded for all groups. At ten hours postinfusion or twelve hours postburn all animals were given water ad lib. Twenty-four hour postinfusion hematocrit values were then obtained on the animals that survived.

A study of the hematocrit data presented in table 2 shows the following:

1. Among animals receiving the same therapy surviving rats showed a lower average hemoconcentration than those that died.

2. In comparing different therapeutic regimes animals infused with red cells

in physiological saline solution, normal whole blood or hemoglobin solutions showed a greater average hemoconcentration than the untreated controls or any of the other groups.

TABLE 2

Average hematocrit values as per cent of preburn hematocrits for various infused groups

2 per cent body weight infused—1 infusion

INFUSION FLUID	POSTBURN PRE-INFUSION		POSTINFUSION					
	No. of rats	2 hours	0.5 hour	2 hours	5 hours	10 hours	24 hours	
Whole blood	7	117	129	128	133	135	109	Survived
	1	125	147	139	156	134	144	Died
Plasma	12	128	110	117	118	112	98	Survived
Human serum albumin Cohn —25 per cent	9	125	102	112	121	124	103	Survived
	1	125	104	124	132			Died
Saline 0.85 per cent	7	134	133	123	124	127	94	Survived
	1	142	109	134	124			Died
Albumin 5 per cent	8	130	110	120	127	127	107	Survived
	1	119	98	122	134	130		Died
Knox P-20 gelatin 4 per cent solution	9	125	100	108	114	121	103	Survived
	1	131	104	114	124	126	145	Died
Red blood cells in 0.85 per cent saline	8	126	144	143	141	149	121	Survived
	2	128	149	151	157	156	162	Died
Oxypolygelatin 37°C. 5 per cent solution Pauling and Campbell	8	126	102	111	119	121	102	Survived
	2	130	102	111	124	131		Died
Human hemoglobin 7 per cent solution Sharp and Dohme	6	127	109	123	135	131	118	Survived
	4	134	109	126	133	153		Died
Oxypolygelatin 22°C. 5 per cent solution	3	117	104	109	112	117	104	Survived
	7	126	100	110	123	124	111	Died
Controls—untreated	13	117	126	125	123	123	108	Survived
	15	124	124	130	135	134	138	Died
Rat globin 1.5 per cent—Strumia	2	127	122	123	106	103	78	Survived
	3	129	138	129	117	126	114	Died

3. Surviving animals receiving plasma began to reconstitute their normal cell-plasma ratio levels between five and ten hours postinfusion. The other groups did not begin to reconstitute this normal cell-plasma ratio until after ten hours postinfusion, at which time water was given.

Comparative efficacy of 4 per cent and 10 per cent of the body weight replacements in 100 per cent lethal burns. The data presented in table 1 suggested that rats subjected to scald burns according to the technique outlined above were not in critical need of colloid replacement since they were effectively treated by physiological saline solution. In order to determine whether this form of therapy is adequate regardless of the severity of injury or only within limited degrees of trauma, the experimental conditions were altered to produce a 100 per cent mortality in the untreated control animals. The experimental conditions were maintained as outlined above with the exception that the length of the immersion time was increased from fifteen seconds to thirty-five seconds. A total of twenty-four rats were burned for thirty-five seconds and received no treatment. All were dead within twenty-four hours (table 3).

TABLE 3

Postburn survival rates for various infused groups of rats burned at 90°C. for 35 seconds

INFUSION FLUID	NO. OF RATS	PER CENT SURVIVAL POSTINFUSION					
		8 HOURS	12 hours	24 hours	48 hours	72 hours	10 days
Untreated controls	50	60	10	0	0	0	0
4 per cent body weight infused—2 infusions							
0.85 per cent NaCl	14	100	86	29	29	29	29
Human serum albumin 5 per cent solution*	14	100	93	86	86	86	86
Knox P-20 gelatin 4 per cent solution	14	93	36	0	0	0	0
0.8 per cent body weight infused—2 infusions							
Human serum albumin 25 per cent solution	4	100	50	0	0	0	0
10 per cent body weight infused—3 infusions							
0.85 per cent NaCl	14	100	100	86	86	86	86
Human serum albumin 4 per cent solution	14	100	100	93	93	86	86
Knox P-20 gelatin 4 per cent solution	14	100	79	64	57	50	50
1.6 per cent body weight infused—3 infusions							
Human serum albumin 25 per cent solution	4	100	50	0	0	0	0

* Diluted from 25 per cent stock solution containing 0.3 M NaCl with "physiological saline", the precise content of which was not analyzed. Later analysis of samples from the same source showed a considerable range of variation.

Three groups of fourteen rats each were subjected to the thirty-five second burn, and each group was infused postburn with one of the following fluids: 0.9 per cent physiological saline solution, 4 per cent Knox P-20 gelatin, and 5 per

cent human serum albumin solution diluted from the 25 per cent stock solution with physiological saline. A fourth group of four rats was burned in a similar manner and infused after the burn with one-fifth the volume of a 25 per cent albumin solution.

All rats in each of the first three groups were infused with 2 per cent of their body weight at two hours after the burn and 2 per cent of their body weight at five hours after the burn, a total of 4 per cent of their body weight. All rats in the fourth group, receiving the 25 per cent albumin solution, were infused with 0.4 per cent of their body weight at the same intervals or a total of 0.8 per cent. All animals were given water ad lib at twelve hours postburn.

Hematocrit samples were usually obtained from half of the rats in each group at the following intervals: pre-burn, two hours postburn, pre-infusion and five minutes postinfusion; five hours postburn-pre-infusion and five minutes postinfusion and ten hours postburn (table 4).

Table 3 shows the survival percentages at intervals for a ten day period. These survival percentages showed no changes between twenty-four hours and ten days. Twenty-nine per cent of the saline-infused rats survived; 86 per cent of the 5 per cent albumin-infused rats survived; none of the 25 per cent albumin-infused and none of the gelatin-infused animals survived.

The efficacy of the 5 per cent solution of human serum albumin in these experiments showed a significant improvement in the survival rate compared to the 25 per cent albumin solution, physiological saline solution and Knox P-20 gelatin. Subsequent experiments indicated that the difference between 5 per cent albumin and saline was due to higher sodium chloride concentration in the former.

In view of the results obtained by Tabor and Rosenthal (3, 4) with saline therapy in shock, it was decided to repeat the experiments using larger volumes of the infusion fluids.

A second series of experiments was performed in which three groups of fourteen rats each and a fourth group of four rats were subjected to a thirty-five second burn under conditions similar to those described above. All the rats in each of the first three groups were infused with a total of 10 per cent of their body weight as follows: 4 per cent two hours after the burn; 3 per cent five hours after the burn and 3 per cent ten hours after the burn. One group received 0.85 per cent physiological saline solution; a second group received 4 per cent human serum albumin, and the third group received 4 per cent Knox P-20 gelatin. The rats in the fourth group received a total of 1.6 per cent of their body weight, hence the same gram equivalent of 25 per cent albumin as those infused with the 4 per cent albumin solution. This infusion was administered in three equal volumes at the same time intervals. Hematocrit samples were taken on half of the rats in each group at the same time intervals recorded above (table 4).

Survival percentages are recorded for a ten day period in table 3. With the increased volume of the infusion fluid, survival of the gelatin-treated animals significantly increased from 0 to 50 per cent; survival of the saline-treated animals significantly increased from 29 to 79 per cent. No difference in survival rate

was obtained with the 4 per cent albumin solution, and none of the four rats which received the 25 per cent albumin solution survived. Hence, in this series of experiments no significant difference in survival obtained between the saline

TABLE 4

Average hematocrit values as per cent of preburn hematocrits for various infused groups of rats burned at 90°C. for 35 seconds

INFUSION FLUID	NO. OF RATS	TIME AFTER BURN							END RESULT
		2 hours		5 hours		10 hours		24 hours	
		Pre-infusion	Post-infusion*	Pre-infusion	Post-infusion*	Pre-infusion	Post-infusion*		
Uninfused controls	8	126		125					Died
4 per cent body weight infused—2 infusions									
0.85 per cent NaCl	2	129	128	135	131	120		115	Survived
	5	125	119	126	111	115			Died
Human serum albumin 5 per cent solution	5	118	91	116	81	111		102	Survived
	2	119	93	116	76	103			Died
Knox P-20 gelatin 4 per cent solution	7	116	87	108	77	105			Died
0.8 per cent body weight infused—2 infusions									
Human serum albumin 25 per cent solution	2	129	117	133	119	116			Died
10 per cent weight infused—3 infusions									
0.85 per cent NaCl	8	123	95	122	81	112	84	91	Survived
	3	117		128		118			Died
Human serum albumin 4 per cent solution	6	121	91	114	67	104	65	94	Survived
	2	112	91	110	86	103	71	95	Died
Knox P-20 gelatin 4 per cent solution	3	111		107	97		76		Survived
	4	119	83	100	81	96	79	79	Died
1.6 per cent body weight infused—3 infusions									
Human serum albumin 25 per cent solution	2	120	82	111	90	118	108		Died

* 5 minutes postinfusion.

and the 4 per cent albumin-infused animals, but both of these show a statistically significant improved survival rate when compared to gelatin. In this series, as in the former, a significant difference in survival was obtained between the diluted

and concentrated albumin solution. In these experiments the results indicate that the colloid is of limited value without concomitant administration of an adequate salt and fluid volume.

Examination of the hematocrit data recorded in table 4 for these two series of experiments indicates the following:

1. Saline-treated surviving animals receiving an infusion of 4 per cent of the body weight had hemoconcentration percentages similar to the untreated controls which died. All other infusion fluids in these experiments of both surviving and dead animals produced greater hemodilution than the untreated control animals. Hence, it may be concluded that hemoconcentration per se is not responsible for death in these experiments.

2. Significant hemodilution values following saline infusion were obtained only after the larger infusion volumes (10 per cent of the body weight).

3. Rats infused with gram equivalents of concentrated albumin solution (25 per cent) were unable to maintain hemodilution values equivalent to those of rats infused with diluted albumin solution (4 or 5 per cent).

4. The hematocrit data seem to offer no explanation as to the cause of the ineffectiveness of the gelatin infusions in promoting survival. Hemoconcentration can be ruled out as a contributing factor since at most intervals the hematocrit percentages for the gelatin-infused animals were consistently lower than those of the other groups.

Comparative efficacy of 4 per cent of the body weight infusions in 100 per cent lethal burns of solutions containing different concentrations of sodium chloride alone and in combination with other ions and colloid. The results of the experiments listed in table 3 with 4 per cent replacements showed significantly improved survival rates of the 5 per cent albumin-treated animals compared with those receiving physiological saline solution. The 5 per cent albumin solution was prepared by diluting one volume of the 25 per cent solution (in 0.3 molar sodium chloride) with four volumes of a 0.9 per cent sodium chloride solution. The resulting saline concentration of the infused 5 per cent albumin solution was 1.06 per cent. Since no animals had been infused with a hypertonic saline solution (1.06 per cent) and since experimental evidence (table 3) showed that an increased volume of a 0.85 per cent sodium chloride solution was as effective in promoting survival as the 5 per cent albumin solution, it seemed necessary to compare the hypertonic saline solution simultaneously with the 5 per cent albumin solution containing 1.06 per cent sodium chloride.

In the last group of experiments (table 5) the sodium chloride concentration of 5 per cent albumin was made up to 1.06 per cent on the assumption that the 5 per cent albumin used in the experiments summarized in table 3 had been diluted with 0.9 per cent sodium chloride. Actually the diluent was physiological saline solution as prepared for hospital use, and subsequent analysis of a number of samples from the same sources showed considerable variations in concentration. This may have been a factor in the discrepancy between the 86 per cent survival found (table 3) and the 60 per cent survival found (table 5).

In order to try to separate further the effects of the sodium chloride per se,

a salt-poor albumin was tested in rats subjected to the 100 per cent lethal burn. Four groups of animals were tested in experiments conducted simultaneously according to the procedure described above. One group received a 5 per cent solution of albumin which was prepared by diluting one volume of the 25 per cent salt-poor stock solution (containing 0.3 gram of sodium ion per 100 cc.) with four volumes of 0.9 per cent sodium chloride solution. The resulting sodium chloride concentration of the 5 per cent solution was 0.87 per cent. A second group, serving as a sodium chloride control group for the first, received 0.85 per cent sodium chloride solution. A third group received a 5 per cent albumin solution in dextrose. This solution was prepared by diluting one volume of the 25 per cent salt-poor stock solution with four volumes of a 6 per cent dextrose solution. The resulting sodium ion concentration was 0.06 per cent. The fourth group, serving as a control for the third, received a 6 per cent dextrose solution.

TABLE 5

Postburn survival rates of various infused groups of rats burned at 90°C. for 35 seconds

4 per cent bodyweight infused—2 infusions

INFUSION FLUID	NO. OF RATS	PER CENT SURVIVAL					
		8 hours	12 hours	24 hours	48 hours	72 hours	10 days
5 per cent albumin* in 1.06 per cent NaCl solution	20	75	70	60	60	60	60
1.06 per cent NaCl solution	20	85	70	60	55	55	55
5 per cent albumin* in 0.87 per cent NaCl solution	20	80	55	30	30	30	30
0.85 per cent NaCl solution	20	55	40	35	25	25	25
5 per cent albumin* in 6 per cent glucose solution	14	9	0	0	0	0	0
6 per cent glucose solution	7	0	0	0	0	0	0
Citrated rat plasma	14	72	50	36	29	29	29
Ringer-Locke K-free NaCl (9.35 gm.) $CaCl_2 \cdot 2H_2O$ (0.24 gm.) $MgCl_2 \cdot 6H_2O$ (0.005 gm.) dextrose (0.5 gm.) $NaHCO_3$ (0.5 gm.) H_2O (1000 cc.)	20	70	40	30	30	30	30
Kreb's dextrose Ringer NaCl (6.95 gm.), KCl (0.35 gm.) $CaCl_2 \cdot 2H_2O$ (0.37 gm.) dextrose (1 gm.) $NaHCO_3$ (2.1 gm.) Sorensen phosphate (0.16 gm.) $MgSO_4 \cdot 7H_2O$ (0.3 gm.) H_2O added to make 1000 cc	14	71	36	29	29	29	29
NaCl (8.7 gm.) $CaCl_2$ (0.37 gm.) $MgCl_2 \cdot 6H_2O$ (0.2 gm.) H_2O 1000 cc	14	57	43	29	29	29	29
Untreated controls	48	59	11	0	0	0	0

* Human serum albumin.

The survival percentages of these four groups (table 5) show no significant difference in survival: *a*, between the group of animals which received 5 per cent albumin in 0.87 sodium chloride (30 per cent) and their controls which received

0.85 sodium chloride (25 per cent), nor *b*, between the animals which received 5 per cent albumin in 6 per cent dextrose and their controls, which received 6 per cent dextrose solution, both showing a survival percentage of zero. However, the rats which received a 5 per cent solution of albumin in 0.87 per cent sodium chloride had a significantly higher survival rate (30 per cent) than those which received a 5 per cent albumin solution containing 0.06 per cent sodium ion (zero survival).

Since these results clearly indicated that under the conditions of these experiments colloid (albumin) without sodium chloride would not promote survival significantly and that colloid added to saline was of no greater benefit than saline alone, the question arose concerning the possible enhancement of the value of saline when in combination with other ions.

Using the same procedure employed in the above groups, three solutions of various ionic components and concentrations were compared with citrated rat plasma.

The results of these experiments (table 5) showed no significant differences in survival percentages of any of the four solutions compared to those of the rat plasma or the physiological saline solution.

Hematocrit values, as per cent of pre-burn hematocrits, are recorded in table 6 for those burned rats which received the infusion fluids listed in table 5. In most experiments half of the rats in each group were subjected to hematocrit sampling. Of these, the average hematocrit values for those that survived and those that died have been recorded separately. Only two values for untreated controls were obtained because of the early demise of these animals.

From table 6 the following general trends of the hematocrit following this degree of injury and subsequent infusion can be noted:

1. Postburn, postinfusion values for all surviving rats were lower than the postburn value for the controls.

2. The difference between the values for survivors and dead animals in most groups was negligible.

3. In both surviving and dead groups albumin-infused animals showed consistently greater hemodilution than their respective controls.

4. No consistent correlation between survival and hemoconcentration was obtained.

Discussion. The Wistar rat is particularly well suited to the study of one major criterion, namely "survival", under strictly standardized conditions of procedure. Numbers sufficient for statistical analysis can readily be obtained. However, in the rat studies of blood chemistry are limited by the difficulty of obtaining blood samples; likewise, physiological data are limited by the influence of necessary manipulation upon survival time.

In order to make full use of the principal advantage of this species, namely survival, only one additional criterion was employed in this study—hemodilution. Considered as an approximation of directional changes in plasma volume hematocrit determinations were made in half of the animals in most groups. The survival rates were significantly decreased by the manipulation of the animals

and the withdrawal of a total of approximately 0.06 cc. of blood from the tail vein.

TABLE 6

Average hematocrit values as per cent of preburn hematocrits for various infused groups of rats burned at 90°C. for 35 seconds

4 per cent body weight infused—2 infusions

INFUSION FLUID	NO. OF RATS	TIME AFTER BURN						END RESULT
		2 hours		5 hours		10 hours	12 hours	
		Pre-infusion	Postinfusion**	Pre-infusion	Postinfusion**			
Uninfused controls	8	126		125				Died
5 per cent albumin* in 1.06 per cent NaCl solution	4	118	83	107	78	99	97	Survived
	6	121	74	109	73	103		Died
1.06 per cent NaCl solution	4	117	111	119	110	109	93	Survived
	6	122	112	118	110	100		Died
5 per cent albumin* in 0.87 per cent NaCl solution	2	121	91	113	85	105	108	Survived
	8	121	88	120	82	106	111	Died
0.85 per cent NaCl solution	2	113	111	111	104	111	100	Survived
	5	115	107	118	101	115	115	Died
5 per cent albumin* in 6 per cent dextrose solution containing 0.06 per cent Na ions	7	122	90	124	74			Died
6 per cent dextrose solution	3	118	106	111	79			Died
Citrated rat plasma	1	120	95	124	89	114	96	Survived
	5	116	90	114	79	106		Died
Ringer-Locke K-free	4	125	107	122	102	108	99	Survived
	3	120	111	116	105			Died
Kreb's dextrose Ringer	1	123	112	119	106	110		Survived
	6	134	122	130	117	132		Died
NaCl (8.7 gm.) $CaCl_2 \cdot 2H_2O$ (0.37 gm.) $MgCl_2 \cdot 6H_2O$ (0.2 gm.) H_2O 1000 cc.	1	118	102	110	102	110	90	Survived
	4	113	104	120	100	106		Died

* Human serum albumin.
** 5 minutes postinfusion.

The injection rate employed in unanesthetized rats is usually greater than that commonly employed in the dog or clinically in patients. The more rapid rate

of injection may detract from the efficacy of certain solutions, such as gelatin and 25 per cent albumin or the erythrocyte derivatives, globin and hemoglobin. In any case, rapid alterations in fluid distribution, cardiac output, and peripheral circulation resulting from rapid injection of osmotically active colloid solutions known to be more viscous than plasma or known to produce vasomotor reactions, may be less beneficial than the slower compensations following slow rates of infusions.

In comparing the therapeutic response of rats with that of dogs or patients to fluid replacement following thermal injury, one species variation might be considered, i.e., changes in body temperature. The rectal temperatures of a few rats (not included in the survival data) subjected to the procedure employed in this study declined as much as 10° to 12°F. This is in striking contrast to the increases in body temperature of dogs and man following thermal injury involving such a large percentage of the body surface. Possibly because of the physical property of viscosity, gelatin solutions may be less effective where there is a decline in body temperature. Viscosity of the gelatin solution is markedly increased by a drop of 10°F., which would tend to increase resistance to blood flow in small caliber vessels, such as the capillaries.

The above factors are mentioned since they are thought to be pertinent to an attempt to correlate results of these studies in the rat with those of other published results on mice (4), dogs (5, 6), and patients (7). Our results from these rat studies in general are in confirmation of those of *a*, Rosenthal (4) who employed mice with survival rates as the major criterion of comparative effectiveness of saline, serum, etc.; *b*, earlier published results (5) using hemodilution, blood pressure and survival in dogs rapidly infused at intervals with saline, gelatin (Knox B78-6 per cent) and plasma; *c*, Ely and Angelo (8) who used hemodilution as the criterion in burned rabbits injected with physiological saline solution, gelatin-saline-glucose, and plasma.

The results of the present study are in agreement with the clinical studies of Evans and Rafal (7) in burned patients infused with Knox P-20 gelatin only in so far as comparable criteria were employed, namely, measures of increased plasma volume or hemodilution. Gelatin (Knox P-20) was reported by Evans to be as effective as plasma in burned patients.

Hemoconcentration can be correlated with the survival time and survival rates of the untreated animals. However, the degree of hemodilution following the various infusions is not well correlated with the survival time or rates. The colloidal solutions which provide the greatest degree of hemodilution are not necessarily the most effective in terms of survival of rats subjected to severe burns. Although the colloids in saline may compensate osmotically for the loss of circulating plasma protein, survival rates may be equivalent or superior with saline alone even though the hemodilution and apparent increment in blood volume are often temporary or negligible. As in the dog (5) hemodilution is of limited prognostic value following fluid therapy. Whole blood or erythrocytes in saline infusions may result in extremely high hematocrit values in animals which survive.

In these latter cases, however, it should be pointed out that hemoconcentration is associated with *a*, a compensatory increase in total circulating blood volume, the cells of which are not readily lost through the endothelium of the injured blood vessels, and *b*, a corresponding increase in oxygen carrying capacity per unit of blood flow through peripheral capillaries, the effective resistance of which would theoretically be increased with the increase of viscosity due to hemoconcentration.

In the present study 4 per cent Knox P-20 was less effective in the 100 per cent lethal burn than physiological saline solution or 5 per cent human albumin solution as shown by the survival rates. Dunphy and Gibson (9), using bovine albumin solution as a therapeutic agent in the treatment of burns in dogs, found albumin less advantageous than plasma. Their criteria of effectiveness and conclusions were largely based upon hemodilution, plasma volume, and pathological studies. Survival rates were not employed as the animals were used for tissue study at a suitable interval after the burn.

If we are justified in the assumption that the survival of the rat subjected to a burn lethal to 100 per cent of untreated controls is the result of the successful compensation for induced deficiency factors which otherwise lead to death, then it may reasonably follow (from the data on various replacement fluids with and without certain ions, colloid or blood cells) that the major limiting deficiency factors compensated for by the various fluids including plasma and whole blood are sodium chloride and water. The survival rates following the larger volumes of saline infused are as favorable as with any of the other fluids employed separately or in combination with other components of whole blood. The inclusion of other ions of plasma, as in modified Ringer solutions, did not significantly enhance the value of physiological saline solutions. The increase in survival with 1.06 per cent saline, as compared with 0.85 per cent, is on the borderline of statistical significance ($P = 0.05$). The deficiencies of plasma colloids, due to leakage into the injured area, and of erythrocytes resulting from thermal hemolysis are apparently not of a critical order of magnitude providing the deficiency in extracellular fluid is corrected by adequate sodium chloride and water. Plasma or colloidal plasma substitutes in saline or glucose resulted in survival rates statistically similar or directionally inferior to physiological saline solution.

The sodium ion is well known to be the chief extracellular cation responsible for the osmotic equilibrium of fluid between cells and the extracellular compartment including the blood stream, whereas potassium holds a similar key position within cells (10). When sodium diffuses into tissue cells of the injured area, as has been reported (11, 3, 12), a deficit in extracellular sodium ions is believed to occur. Our experiments indicate that this deficit is critical and requires the replacement of displaced sodium and water with large volumes. Fluid, as dextrose solution by vein, was of little value without the sodium ion for its proper distribution in the body fluid compartments. Intravascular injection of colloid without sodium, i.e., salt-poor albumin diluted with dextrose solution, was likewise of limited or no value.

SUMMARY

1. Homologous plasma, whole blood, erythrocytes (suspended in physiological saline solution), and globin (modified); human serum albumin and human hemoglobin solution; sodium chloride solution, 0.85 per cent; gelatin, oxypolygelatin, sodium chloride, 1.06 per cent; 6 per cent dextrose; and three modifications of Ringer's solution were compared for their effect on the survival of rats subjected to carefully standardized scald burns. The albumin was infused in 4 per cent, 5 per cent and 25 per cent concentration, and the 5 per cent concentration was prepared with three concentrations of sodium chloride in order to evaluate the relative importance of salt and water in albumin solutions. The comparative effectiveness of these agents is shown in the tables.

2. The results indicate that the effectiveness of albumin solution and the other infusion fluids which afforded the highest survival rates could be attributed to the sodium salts and water content.

3. Physiological saline solution, although comparatively ineffective in correcting hemoconcentration, was found to be as effective as any of the various infusion fluids employed in promoting the survival of rats subjected to scald burns.

4. In general hemoconcentration was somewhat greater in animals which succumbed than in those which survived. However, the cell:plasma ratio was not of value in the prognosis of individual animals nor in comparing infusion fluids. The solutions which provided the most hemodilution did not always promote higher survival rates, whereas many animals with severe hemoconcentration in certain of the infusion groups did survive.

5. Hemoconcentration and plasma volume do not appear to be critical factors in the survival of the rats after thermal injury of the type produced.

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BIOASSAY OF STEROID HORMONES USING AQUEOUS SODIUM LAURYL SULFATE SOLUTION AS THE DISPERSING AGENT[1]

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In studying the distribution and fate of the steroid hormones in biologic systems, it became necessary to use bioassay in the determination of estradiol. Twombly and Taylor (1), who studied the inactivation of estradiol by the liver and were therefore confronted with a similar problem, used the method of Lauson, Heller, Golden, and Sevringhaus (2), which is based on the increase in weight of the immature rat's uterus. An examination of the data of the first named authors revealed a wide variation in uterine weight increase in different strains of rats; more impressive still was the fact that the maximum uterine weight attained never reached the maximum which we had observed in the bioassay of chorionic gonadotropin (3), and the increase of uterine weight with increase in dosage was far more gradual for the estrogen than for the gonadotropin. The bioassay of the gonadotropin, using the immature rat uterus as the test object, is an indirect test in which the gonadotropin stimulates the ovary to produce the estrogen which in turn develops the uterus. Since the isolation of estradiol from the ovary, it is generally assumed that estradiol is the ovarian hormone par excellence (4). If this assumption is correct, it should be possible to produce the same effects by dosage of estradiol as are produced by the gonadotropins. Either the assumption is incorrect or some other hormone enters the picture. In this laboratory (5) we have repeatedly demonstrated the importance of delayed resorption or divided dosage in simulating the physiologic state.

In this paper we report on the marked effect which divided dosage or delayed resorption has upon the physiologic response to estradiol. To produce delayed resorption, sodium lauryl sulfate (S.L.S) (6), which effectively delays the resorption of the gonadotropins, has been used. It has the advantage of producing a stable aqueous colloid. It was necessary in assaying steroids present in biologic systems to control the augmentation produced by inert substances of which the higher fatty acids are known to have a marked influence (7).

Since the androgens also stimulate uterine hypertrophy, and since assays based on seminal vesicle increase have not been very satisfactory for the androgens, the influence of delayed resorption from aqueous S.L.S. solution has been applied to testosterone.

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We wish to thank Dr. Erwin Schwenk of Schering Corporation for the steroid hormones used in these experiments. These hormones were of the highest purity, the alpha estradiol melting at 176.8–177.1°C. (cor.) and the testosterone melting at 153.7–154.2°C. (cor.).

RESULTS. *Estradiol.* In table 1 a comparison of number of doses per day (one, two, or three) and vehicle (either isotonic saline or 0.5 per cent S.L.S. in 0.9 per cent NaCl solution) is made. Each value is the mean of values for eight to ten rats weighing 40 to 55 grams terminally. Dosage was begun the 22nd or 23rd day of age and continued for three consecutive days. The uteri were weighed 76 hours after initial dosage. In all cases the volume of a single injection was 0.1 cc. When two doses per day were given, the second was eight hours after the first. Three doses per day were given at eight-hour intervals. At the lowest effective dose (0.03 γ total), delayed resorption is without significant influence. At the range of 0.15 γ total, divided dosage significantly increases the response. At the range of 0.3 γ total, delayed resorption, which is the equivalent of multiple division of doses, further increases the response. Thus one dose per day in saline produces a uterine weight of 59 ±1.5, while

TABLE 1

Effect of divided dosage upon the uterine response of immature rats to the administration subcutaneously of estradiol in aqueous saline or aqueous 0.5 per cent sodium lauryl sulfate solution

TOTAL DOSE IN GAMMA	MEAN UTERINE WEIGHT IN MGM. PLUS STANDARD DEVIATION OF MEAN OF GROUPS OF 8 TO 10 RATS BEGUN AT 22–23 DAYS OF AGE					
	When administered in saline			When administered in S.L.S.		
	once per day	twice per day	thrice per day	once per day	twice per day	thrice per day
0.6	68 ± 2.4	90 ± 2.7		83 ± 2.9	95 ± 2.4	
0.3	59 ± 1.5	76 ± 2.6	94 ± 2.1	73 ± 3.5	89 ± 3.2	102 ± 2.7
0.15	47 ± 1.0	63 ± 3.0	68 ± 1.9	59 ± 2.8	63 ± 2.6	74 ± 3.2
					*60 ± 2.8	
0.06	28 ± 0.7			33 ± 1.3	35 ± 1.7	
0.03	22 ± 0.8			24 ± 1.1	23 ± 1.5	
0.0	18 ± 0.6					

* Plus 25 per cent human serum.

three doses per day in S.L.S. solution produce a uterine weight of 102 ± 2.7 mgm. For all values the standard deviation of the mean is less than 6 per cent of the mean organ weight. The effective assay dosage is from 0.03 to 0.3 γ estradiol. With the pure hormone, the assay procedure using one dose per day in saline is as accurate as multiple dosage in S.L.S. because of the small variation in response, even though the weight increase curve is not as steep. The use of two doses per day, with 0.5 per cent S.L.S. as the vehicle, is the procedure recommended for practical purposes provided the effective range does not exceed 0.15 γ estradiol. In this range there is no significant difference between one and two doses per day in S.L.S. and two doses per day in saline, so that impurities which would delay resorption are controlled. Using eight to ten rats per dosage level, the accuracy of the assay is within 15 per cent 19 of 20 times.

In eight trial bioassays between March 16 and May 28, 1947, the standard

given was a total dose of 0.15 y per rat. On the basis of eight to ten rats per assay per standard the results using the assay curve given in the graph were as follows: 0.176 ± 0.008, 0.140 ± 0.010, 0.175 ± 0.020, 0.145 ± 0.010, 0.150 ± 0.008, 0.130 ± 0.006, 0.140 ± 0.008 and 0.150 ± 0.004. In six of these eight values the deviation from the amount given approximates once the standard deviation of the mean. In performing a bioassay, a standard is always performed simultaneously with the unknown and the division of rats is by litter mates. Any deviation of response from the standard curve outside the normal variation would thus be detected. The use of litter mates increases the accuracy of the assay. Correlation coefficients calculated for series of ten and twelve pairs of rats, in which the standard deviation of the mean was high, viz., 5 and 6 per cent of the control mean, and in which there was therefore a rather wide spread in response, gave values for r[3] of 0.85 ± 0.09 and 75 ± 0.16. Within the body weight range used, the correlation between uterine weight response and body weight was nil, viz., r = 0.08 ± 0.24 for eighteen rats.

Testosterone. Hays and Mathieson (8) and Greene and Burrill (9) have used the immature male rat as the test object for the assay of testosterone propionate in oil. In experiment 1, the procedure of Hays and Mathieson shows no significant weight increase of seminal vesicles for testosterone. In experiments 2, 3, and 4, the dosage was repeated three times and the period of action was extended. Again the results were highly unsatisfactory. Since Freeman and Small (10) had found that the uterus of the immature rat was the organ most appropriate for the assay of testosterone propionate in oil, uterine response to testosterone was tested in experiment 5. The results were not encouraging.

Oil as a vehicle was then abandoned and 1 per cent S.L.S. in aqueous saline solution was used according to the schedules given in the table for experiments 6, 7, 8, and 9. Experiment 6 was designed to establish whether the rats should be killed 24 or 48 hours after the last dose. The result showed no significant difference. Experiment 7 shows that the weight of the test animal has a profound influence upon the response. The response in S.L.S. is far greater than that from sesame oil. However, the results show that as an assay procedure the method is unsuitable as the weight increase reaches a plateau at too low a level. The same holds for the uterine assay in experiments 8 and 9.

In the case of estradiol one dose per day in oil produced the same effect upon uterine hypertrophy as two doses per day of an aqueous solution (Lauson et al. (2)). This relation is not noted in the assay of testosterone by seminal vesicle response, which is negligible in the case of oil and marked on the same total dose

[2] Unpublished data from this laboratory indicate that a 1 per cent solution of castile soap is approximately as effective as a 0.5 per cent solution of S.L.S. in effecting augmentation of sheep pituitary gonadotropins. The injection areas show evidence of inflammation, edema, and necrosis.

[3] r = correlation coefficient Std. error of r =

$$r = \frac{s(xy)}{\sqrt{s(x^2)\cdot s(y^2)}} \qquad \frac{1 - r^2}{\sqrt{n-1}}$$

in S.L.S. colloidal suspension. The uterine response from oil is much less than it is from aqueous solution.

DISCUSSION. Twombly and Taylor (1) give no measures of variability for their assay procedures. The variations observed by Lauson et al. are somewhat greater than those observed by us, but of the same order. These authors point

TABLE 2

Effect of administration of testosterone in sesame oil or aqueous sodium lauryl sulfate under various conditions upon the uterine or seminal vesicle response in immature rats

EXP. NO.	VEHICLE	BODY WT.	NO. OF TEST RATS PER LEVEL	NO. OF DOSES PER DAY	DAYS DOSED	VOL. OF SINGLE DOSE	HRS. KILLED AFTER 1ST DOSE	TEST ORGAN	MEAN WT. OF TEST ORGAN + STD. DEV. MEAN PER TOTAL DOSE OF							
									0 mgm.	0.3 mgm.	0.6 mgm.	0.75 mgm.	0.9 mgm.	1.0 mgm.	1.2 mgm.	1.5 mgm.
1	Sesame oil	43–54	3	1	1	0.2	72	S.V.	5.0	7						
2	Sesame oil	43–60	3	1	3	0.2	72	S.V.	4.5	7	7				8	
3	Sesame oil	46–57	3	1	3	0.2	144	S.V.	4.0	7	8				10	
4	Sesame oil	40–50	8–9	1	3	0.2	120	S.V.†	6.5	8.0	8.5				12.6	
5	Sesame oil	42–50	2–4	1	3	0.2	144	Uterus	15.0	21	23				23	
6	1% S.L.S.	39–56	7–8	1	3	0.25	72	S.V.†				13.7 ±1.7				
	1% S.L.S.	37–49	7–8	1	3	0.25	96	S.V.†	7.4±0.6			14.6 ±1.4				
7	1% S.L.S.	45–60	9–16	1	3	0.5	96	S.V.†	7.1±0.6	17.0±0.8				18.0 ±1.6		26±1.8
	1% S.L.S.	–45	4–19	1	3	0.5	96	S.V.†	6.7	12.6±0.9				15.5 ±0.7		20±1.0
8	1% S.L.S.	40–57	6–10	1	3	0.25	72	Uterus	18.0±1.6	31.0±2.5	40±4.5		47 ±6			
	1% S.L.S.	40–56	6–8	2	3	0.15	72	Uterus	18.0±1.6	23.0±1.0	29±3		29 ±2			
9	1% S.L.S.	40–51	4–5	1	3	0.5	96	Uterus		23	48		54			

† Seminal vesicles + coagulating gland.

out that the method of Astwood (11) is less accurate for small numbers of animals than their own. Their conclusion that the Astwood method would be more accurate than their own for large numbers of animals is hard to reconcile with the well known statistical concept that the error is inversely proportional to the square root of the number of animals employed.

Astwood (11) gives the standard deviation of the mean for six values de-

termined on 300 rats. Assuming roughly 50 rats per dosage level, his experimental error in the effective range (calculated for three levels in terms of the higher level as 100 per cent) varies from 11 to 22 per cent for twice the standard error($\sqrt{d_1^2 + d_2^2}$). Our results for one dose per day in saline and two doses per day in S.L.S. based on 8 to 10 rats per dosage level are of the same order, slightly less rather than greater. Our method is therefore at least twice as accurate as the one described by Astwood. In the method of Astwood the effective weight increase is only 50 per cent, which is approximately 8 to 9 mgm. in absolute weight. It is our experience that the error involved in dissection, blotting, and evaporation while weighing, could hardly be considered less than 1 mgm., so that the inherent weakness of the method is the error of 12 per cent (minimum, larger as the dose decreases) introduced in each single determination. While this error would compensate in a large series of determinations, it is necessary to use five times the number of animals that we use. In our method, the error of dissection and weighing is only 2 to 3 per cent at the high effective dose. Another disadvantage in the method of Astwood is the difficulty of introducing 0.1 cc. of oil by injection. In contrast to aqueous solution, oil has a tendency to leak at the injection site. Deanesly and Parkes (12) objected to oil as a vehicle for bioassay as early as 1933, their main conclusion being that the resorption of the hormone from oil was too irregular.

In figure 1 are plotted the values of uterine response to estradiol administered in aqueous solution in a single daily dose and in aqueous S.L.S. solution once, twice, and thrice daily. The marked effect of delayed resorption and effectiveness of the uniform resorption at a low level is strikingly illustrated; the maximum difference between slow and rapid resorption is over 400 per cent of the minimum amount required. In this graph are superimposed assay data for the chorionic gonadotropin, administered in daily doses for 3 days; in this assay, uteri are weighed 72 hours after initial dosage in rats the same age as those used for the estradiol assay. The data for chorionic gonadotropin are for a total dosage level of 0.01, 0.02, and 0.03 mgm. which produced respectively 26, 63, and 99 mgm. uteri. Superimposing the value of 26 mgm. on the assay graph for estradiol showing maximum response, the remainder of the chorionic gonadotropin curve shows a correspondingly steeper ascent. The conditions of assay for estradiol, while producing the curve approaching the slope obtained for chorionic gonadotropin, have not produced the maximum response. In the case of the sheep gonadotropin, the rapid resorption of hormone from one injection depot will antagonize the effect of the slow resorption from another depot (13). An experiment to ascertain whether an analogous phenomenon existed for estradiol produced negative results. Three-tenths gamma administered once daily for three days in saline solution failed to antagonize the effect of 0.3 gamma administered twice daily in S.L.S. The results of the two series were uterine weights of 92 ± 2.4 versus 89 ± 3.2, in which the latter value is for 0.3 gamma estradiol administered in S.L.S. The experiment was so designed that any fall in uterine weight response, if it occurred, would be in the most sensitive

range of the assay curve. It would therefore appear that the maximum effective response to estradiol is produced solely by a uniform liberation of the hormone.

Since the marked effect of divided dosage or its equivalent delayed resorption, manifested in the dosage range 0.15 to 0.6 y, is not shown in the range of low dosage (at 0.03 y it is nil, at 0.06 y slight) and since there is no evidence for an antagonism phenomenon, one might theorize that estradiol is not rapidly destroyed at the dosage level of 0.03 y, but is rapidly destroyed or removed from action at a higher level.

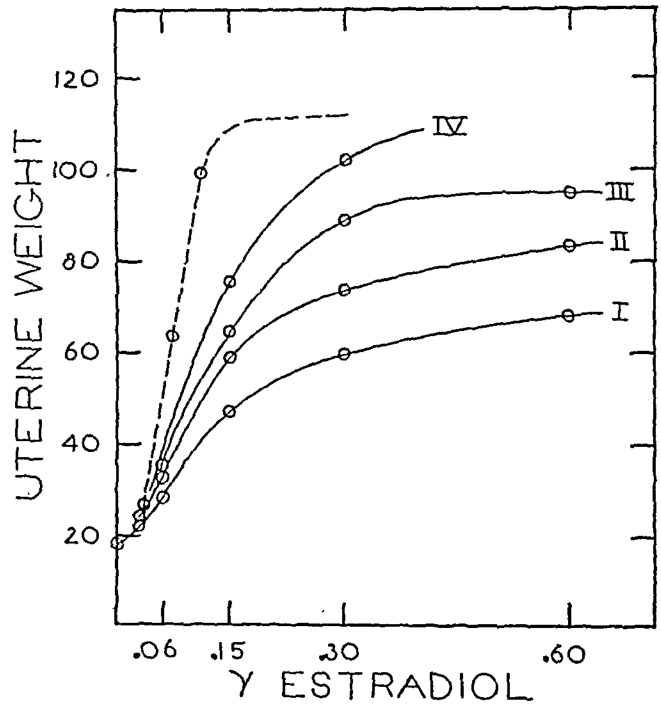

Fig. 1. I Solid line. Administered once daily in saline.
II Solid line. Administered once daily in S.L.S.
III Solid line. Administered twice daily in S.L.S.
IV Solid line. Administered thrice daily in S.L.S.
Broken line illustrates the action of chorionic gonadotropin.
Solid line illustrates the action of estradiol.

SUMMARY

Sodium lauryl sulfate with estradiol or testosterone forms an aqueous colloidal solution which is used on parenteral administration to delay resorption effectively. An assay procedure for estradiol in biologic systems, using sodium lauryl sulfate in aqueous solution as the dispersing agent, is described. With eight to ten rats per dosage level, the accuracy of the assay is within 15 per cent 19 of 20 times. The method controls the influence of certain inert substances which if present might produce augmentation by delaying resorption.

The sensitivity (increase in weight per gamma) and maximum degree of uterine weight stimulation produced by estradiol in the immature rat are mark-

edly affected by delayed resorption. In the experiments recorded a difference of 400 per cent was observed.

The maximum effective response to estradiol is produced solely by a uniform liberation of the hormone. An excess of hormone does not produce antagonism.

The dosage level response curve for estradiol, while approaching that produced by chorionic gonadotropin as the uniform liberation of steroid hormone was more perfectly attained, never reached the efficiency of the response to gonadotropin.

The response to testosterone both for seminal vesicles and uterus is more effective when the vehicle is aqueous sodium lauryl sulfate solution than when the hormone is dissolved in sesame oil. The difference in response is striking.

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OBSERVATIONS ON THE ACTION POTENTIALS INDUCED BY INDIRECT STIMULATION OF SKELETAL MUSCLE IN DESOXYCORTICOSTERONE ACETATE-TREATED RATS ON A LOW POTASSIUM DIET

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In a recent study (Walker, 1947b) it was shown that desoxycorticosterone acetate (DCA) treatment of rats alters the time course of the tension curve and the peak tension of skeletal muscle stimulated with single indirect shocks. It was found that rested muscle of rats treated with DCA shows a marked increase of peak tension, rising time and half falling time over that of normal muscle. This type of response disappears promptly during exercise and reappears when the muscle is allowed to rest.

The purpose of the present study was to investigate the muscle action potentials in DCA-treated rats and to attempt to locate the origin of the repetition revealed in the electrical records. A preliminary report on the repetitive action potentials induced by DCA treatment has been made (Walker, 1947a).

Methods. Immature male rats weighing 100 to 150 grams were used. The animals were prepared for stimulation through the cut sciatic nerve in the manner previously described (Walker, 1947b). Myograms were made either with an isometric lever and optical recording or with a spring lever and kymographic recording. The nerve was stimulated with brief shocks, 3 to 4 times threshold strength. All records of action potentials were made with a condenser-coupled amplifier and cathode ray oscillograph. Muscle action potentials were recorded with leads from 2 steel needles insulated to within 1 mm. of their tips, one being placed in the middle of the belly of the gastrocnemius and the other in the lateral surface of the belly. Endplate potentials were recorded from the soleus. For such recording the insertion of the soleus was dissected free and the muscle was separated from the gastrocnemius to the point of entrance of the soleus nerve and blood vessels. One lead electrode (a pointed capillary tube of glass containing 0.9 per cent NaCl and a fine platinum wire) was placed at the desired point on the belly of the muscle by a micromanipulator. The other lead electrode was inserted into the distal tendon.

To accelerate the rate of diminution of muscle K in the DCA-treated rats a modification of a diet low in K (cf. Miller and Darrow, 1940) was employed as follows: lactalbumin, 26; dexin[1], 24; vegetable fat, 22; sucrose, 25; yeast powder, 2; cod liver oil, 1; NaCl, 1.2; $MgSO_4$, 0.55; Ca $(C_3H_5O_3)_2 \cdot 5H_2O$, 0.6; $CaH_2PO_4 \cdot H_2O$, 1.75; and $FeSO_4 \cdot 7H_2O$, 0.1. The animals on a low K diet

[1] Dextrins, 75 per cent; maltose, 24; mineral ash, 0.25; moisture, 0.75.

were injected intramuscularly with 2 mgm. of DCA[2] each day for 3 to 20 days. Curarization was obtained either with intravenous or with intraperitoneal injection of intocostrin[3]. The KCl, eserine and veratrine injections were always intraperitoneal. Ether anesthesia was used in all experiments.

RESULTS. *Effects of DCA with a low K diet.* A single indirect stimulus 3 to 4 times threshold for the nerve induced an initial large action potential and several subsequent small action potentials in the rested gastrocnemius muscle of DCA-treated rats fed a low K diet. A silent period of variable length, but never less than 6 msec., always followed the initial action potential. The height of the small spikes was about 3 to 5 per cent of that of the initial large spike. The duration and frequency of repetition was not the same in different animals given similar treatment but prolongation of treatment tended to increase repetitiveness (cf. 1A and 2A of fig. 1). The repetition usually persisted over a period of 50 to 100 msec. The repetitive muscle spikes were abolished or reduced in size and number by 3 to 6 responses to shocks at the rate of 1 every 2 seconds (cf. 1B and 2B of fig. 1). Following 5 to 15 minute periods of rest repetition could be again induced with single stimuli. Repetitive discharges were also produced in an animal fed a normal diet (dog chow) and injected with 2 mgm. of DCA daily for 30 days (fig. 1, 1C). In this case likewise the repetition disappeared after a few responses to single stimuli at the rate of 1 every 2 seconds (fig. 1, 2C).

Repetition is increased by the interposition of a second shock shortly after the first. In 1D of figure 1, showing the first double volley to a rested muscle of a rat treated for 3 days with DCA and a low K diet, a greater number of repetitive muscle spikes is seen after the second shock than after the first. The sixth double volley to this muscle showed almost complete disappearance of repetition after both shocks (fig. 1, 2D). Further evidence that repetitive discharge is increased by repetitive stimulation was obtained in myograms recorded from the soleus in an animal given DCA and a low K diet for 15 days. The prolonged falling phase of the tension curve following a single indirect shock in the treated rat (triangles in fig. 2, A) is regarded as evidence of repetitive discharges. The prolongation of the falling phase, particularly in the lower portion of the curve, was exaggerated when 2 successive shocks were delivered with a 4 msec. interval (cf. triangles in B and A of fig. 2). Furthermore, during the slow portion of the falling phase, the tension difference between normal muscle (circles) and DCA-muscle (triangles) is greater following 2 shocks (fig. 2, B) than after a single shock (fig. 2, A).

The repetitive discharges frequently but not always recurred in a somewhat regular rhythm (fig. 1, 2A). In some animals irregular repetition became more regular during the period of discharge (fig. 1, 1E). The time intervals between the discharges increased as the repetitive burst progressed. In some experiments the spike height diminished abruptly toward the end of the period of repetition although the progressive increase of time interval between successive spikes continued uninterrupted (fig. 1, 2E).

[2] The desoxycorticosterone acetate was supplied by the Schering Corporation, Bloomfield, N. J.

[3] The intocostrin was supplied by E. R. Squibb and Sons, New Brunswick, N. J.

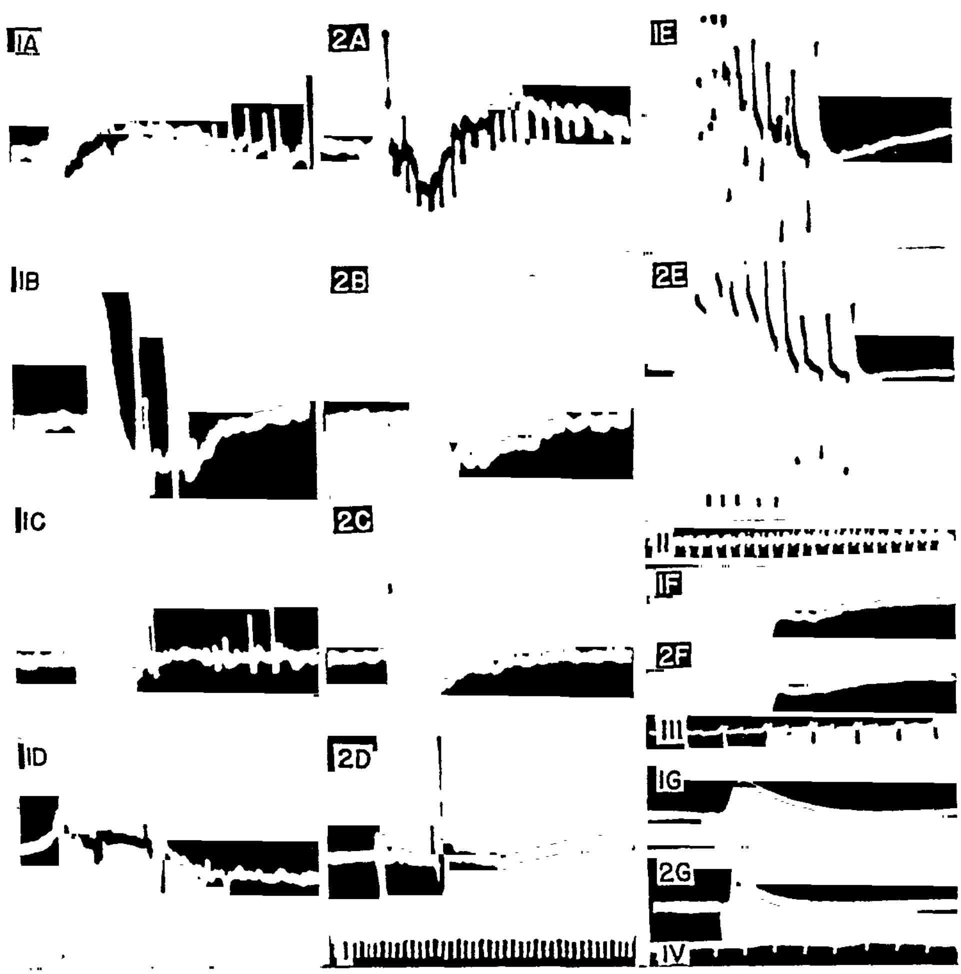

Fig. 1. Records showing the effects of DCA treatment (2 mgm. daily) and a low K diet on action potentials elicited by stimulation of the cut sciatic nerve. 1A to 2E: Gastrocnemius action potentials with the lead electrodes placed in the belly of the muscle. The height of the repetitive action potentials is equal to about 3 to 5 per cent of the height of the initial large action potentials which exceeded the film width. 1F and 2F: Sciatic nerve action potentials with the lead electrodes placed on the nerve distal to the point of stimulation. 1G and 2G: Soleus endplate potentials. Time: 5 msec. intervals. I. Time for 1A to 2D. II. Time for 1E and 2E. III. Time for 1F and 2F. IV. Time for 1G and 2G.

1A. Response to a single shock in a rat treated for 3 days. 1B. First response to a single shock in a rat treated for 4 days. 1C. First response to a single shock in a rat treated for 30 days with DCA alone. 1D. First response to a double volley in a rat treated for 3 days. 1E. First response to a single shock in a rat treated for 4 days, showing asynchronous followed by synchronous repetition. 1F. Nerve action potential in a rat treated for 4 days. First response to a single shock. 1G. Endplate potential of the soleus in a rat treated for 6 days.

2A. Response to a single shock in a rat treated for 20 days. 2B. Same animal, fourth response. 2C. Same animal, fifth response. 2D. Same animal, sixth response. 2E. Same animal, after 10 min. rest. First response to a single shock showing regular rhythm and abrupt diminution of repetition. 2F. Same animal, sixth response. 2G. Endplate potential of the soleus in a normal animal.

(The letters of the 1 series (1A to 1G) of this paragraph should be compared with the corresponding letters of the 2 series (2A to 2G) in the paragraph below.)

The nerve showed single action potentials following single shocks and gave no evidence of repetitive discharges even at an amplification 5 times greater and at a shock strength 4 times greater than those used when records of repetitive muscle action spikes were made. The small background oscillations appearing when the lead electrodes were placed on the nerve were visible both before and after the recording of the action potential (fig. 1, 1F) and they were not abolished by subsequent stimuli (fig. 1, 2F).

The endplate potential of the soleus in the rat as observed after curarization is not more than 10 per cent of the height of the muscle action potential obtained with the same leads before curare treatment. Endplate potentials could be recorded all along the muscle to within a few millimeters of either end. This finding led to the conclusion that the endplates in the rat soleus are widely dispersed. This conclusion was substantiated by examination of histological preparations in which nerve fiber endings were found throughout the muscle with the exception of the extreme tips. The endplate potentials of the soleus in treated animals (fig. 1, 1G) were somewhat more prolonged than those in the normal rats (fig. 1, 2G) but the "slow waves" typical of an eserinized muscle (Eccles, Katz and Kuffler, 1941) were not seen.

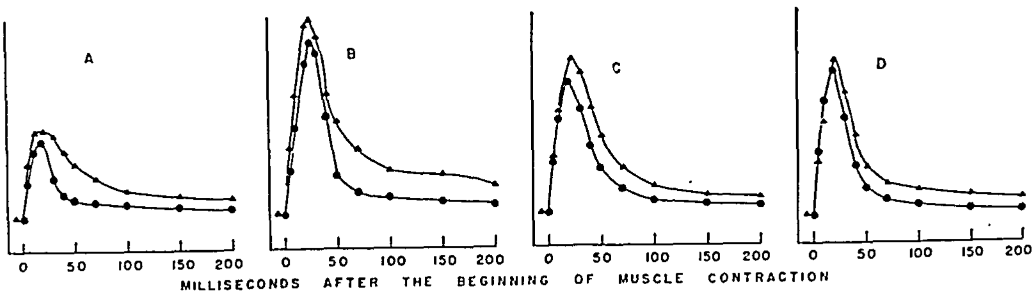

Fig. 2. Reproduction of myograms showing the effect of DCA treatment and a low K diet on the mechanical response of rat muscle. Triangles: treated rats. Circles: control rats. A. Responses of the soleus to single indirect shocks 4 times maximal for the nerve. B. Responses to 2 indirect shocks. Interval between shocks: 4 msec. in treated rat; 5 msec. in control rat. The muscles, the shock strength and the calibration of tension are the same as for A. C. Responses of the gastrocnemius to single indirect shocks 4 times maximal for the nerve. D. Responses of the same muscles plotted in C to single direct shocks, 3 times maximal, in the completely curarized muscle. The calibrations of tension are the same in C and D. The soleus (A and B) was treated for 14 days and the gastrocnemius (C and D) was treated for 8 days.

Effects of curare and K on repetitiveness induced by DCA treatment and a low K diet. Records made at the onset of shallow respiration during the progress of curarization in DCA-treated animals usually showed a slight decrease both of repetition and of mechanical response. The records in 1B and 2B of figure 3 taken 7 minutes after intraperitoneal injection of curare, at a time when respiratory paralysis was clearly in evidence, illustrate the presence of repetition approximately equal to that seen before curare injection in the same animal (fig. 3, 1A and 2A). The amount of repetition was greatly reduced as the depth of

curarization increased (fig. 3, 1C and 2C). In 3 other animals curarization sufficient to reduce the mechanical response about 25 per cent caused complete disappearance of repetitive action potentials. However, tension records obtained from DCA-treated muscle with a series of single direct shocks after complete curarization were similar to the tension records induced by indirect stimulation in the same muscle before curare was given. Both types of stimulation showed a large initial response followed by smaller responses. The initial large mechanical responses of indirectly stimulated muscle were accompanied by repetitive muscle action spikes. Moreover, the large responses of the directly stimulated muscle (triangles in fig. 2, D) were approximately equal to the large

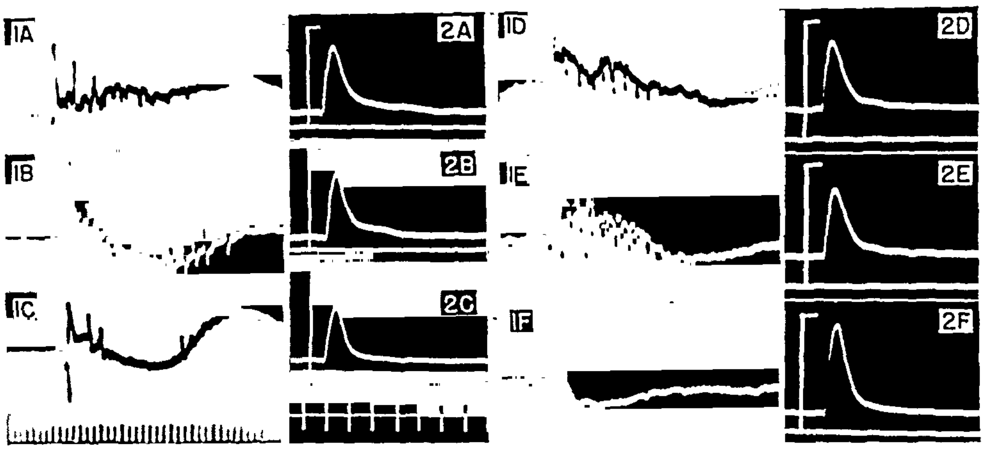

Fig. 3. Effects of curare and K on repetitiveness induced by single indirect shocks in muscle of rats treated with DCA and a low K diet. The 1 columns show muscle action potentials with time marked in 5 msec. intervals. The 2 columns show corresponding myograms with time marked in 50 msec. intervals. The vertical lines subtend 500 grams of tension per gram of muscle as measured from zero tension.

1A and 2A. Low K diet and 2 mgm. of DCA daily for 10 days, before curare. 1B and 2B. Same animal as in 1A and 2A, 7 minutes after intraperitoneal injection of 0.01 cc. of intocostrin per 100 grams of body weight. 1C and 2C. Same animal as in 1A and 2A, 12 minutes after intocostrin injection. 1D and 2D. Low K diet and 2 mgm. of DCA daily for 8 days, before KCl. 1E and 2E. Same animal as in 1D and 2D, 5 minutes after intraperitoneal injection of 80 mgm. of KCl per 100 grams of body weight. 1F and 2F. Same animal as in 1D and 2D, 25 minutes after KCl injection.

responses of muscle stimulated through the nerve before curarization (triangles in fig. 2, C). Furthermore, the prolongation of the falling phase of the tension curve produced by direct stimulation in DCA-treated muscle is about equal to the prolongation induced in the same muscle by indirect stimulation at a time when repetition was shown to be present.

Records obtained 5 to 10 minutes after intraperitoneal injection of KCl showed a slight increase of repetition at a time when the typical potentiating effect of K had not yet appeared (cf. 1D and 2D with 1E and 2E of fig. 2). Fifteen to 25 minutes after the injection of KCl repetitive after discharges were no longer induced by single shocks (fig. 2, 1F) although the developed tension of the muscle was increased as a result of the potentiating effect of K (fig. 2, 2F).

A comparison of the effects of DCA, eserine and veratrine treatment. Simultaneous recording of mechanical and electrical responses from DCA-treated animals fed a low K diet showed that the beginning of repetitive discharges fell within the rising phase of the mechanical response (fig. 4, 1B and 2B). The repetition frequently extended well into the slow slope of the decay of tension appearing in the lower portion of the falling phase. The peak tension was greater and the time course of the tension curve was slower in the initial than in the subsequent

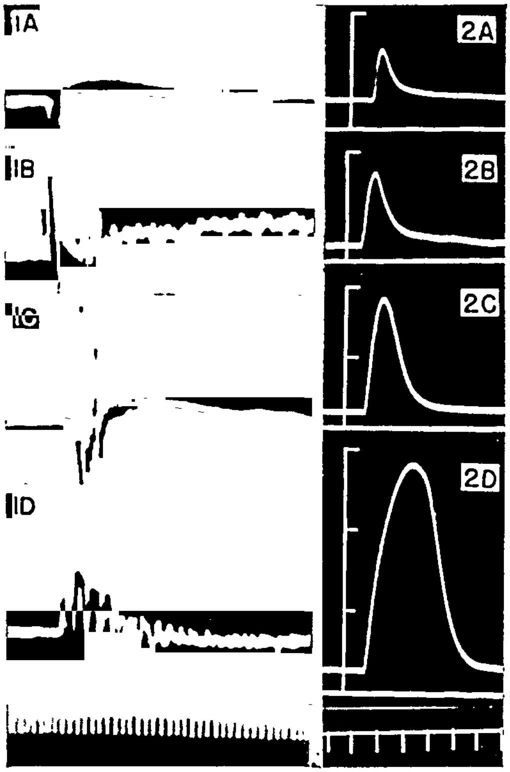

Fig. 4. A comparison of action potentials and myograms of the gastrocnemius in normal, DCA-treated, eserine-treated and veratrine-treated rats. The 1 column shows muscle action potentials with time marked in 5 msec. intervals. The 2 column shows corresponding myograms with time marked in 50 msec. intervals. The vertical lines are divided as units which subtend 500 grams of tension per gram of muscle as measured from zero tension.

1A and 2A. Normal rat. 1B and 2B. Low K diet and 2 mgm. of DCA daily for 15 days. 1C and 2C. Muscle in a rat 15 minutes after intraperitoneal injection of 0.06 mgm. of eserine per 100 grams of body weight. 1D and 2D. Muscle in a rat 15 minutes after intraperitoneal injection of 0.3 mgm. of veratrine hydrochloride per 100 grams of body weight.

muscle responses of a series at 1 every 2 seconds. However, these values were not exaggerated as markedly as in animals fed a normal diet and treated with DCA for 45 to 60 days (Walker, 1947b).

Records from muscle following intraperitoneal injection of 0.06 mgm. of eserine per 100 grams of body weight showed a greater developed tension following single stimuli (fig. 4, 2C) than that induced by similar stimulation in DCA-treated rats (fig. 4, 2B). The action potentials which followed the initial large action potential

of eserinized muscle (fig. 4, 1C) were larger than the repetitive after discharges of muscle in DCA-treated animals (fig. 4, 1B) and the height of these action potentials indicated that a high percentage of the muscle fibers were firing off simultaneously. The repetition began within 5 to 8 msec. after the start of the initial action potential and persisted for only 15 to 25 msec.

For about 15 minutes after the intraperitoneal injection of 0.3 mgm. of veratrine hydrochloride per 100 grams of body weight repetitiveness and tension development in response to single shocks were gradually enhanced. At the point of greatest development of tension (fig. 4, 2D) the appearance of action potentials persisted for about 100 msec. after the onset of repetition (fig. 4, 1D). The first action potential shown after the initial large one gave evidence of synchronous discharge of many motor units. However, as the repetition continued the irregularity of the oscillations appearing in the electrical record indicated the development of pronounced asynchrony. The muscle relaxed, following the cessation of action potentials, about as rapidly as the muscle in a normal animal relaxed following repetitive stimulation. After the initial 15 minute period of enhancement in veratrinized muscle, myograms recorded at successive 5 minute intervals showed a gradual diminution of peak tension and a prolongation of relaxation. The electrical records accompanying these myograms showed a marked decrease in size and number of repetitive action potentials.

DISCUSSION. The results of the experiments with DCA-treated rats raise the question of the origin and nature of the repetitive muscle action potentials. The failure to obtain repetitive action spikes when the recording electrodes were placed on the nerve distal to the stimulating electrodes showed that the repetition in the muscle is not induced by impulses arising at the point of stimulation of the nerve. Indirect evidence that the repetitive discharges can originate independently of motor nerve influence was obtained by direct stimulation of the muscle after complete curarization. The initial large mechanical responses of directly stimulated muscle (triangles in fig. 2, D) were similar to those produced by indirect stimulation of the same muscle (triangles in fig. 2, C) at a time when electrical records showed repetitive muscle spikes. Furthermore, the subsequent smaller mechanical responses resulting from direct shocks repeated at the rate of 1 every 2 seconds resembled the subsequent responses induced by indirect stimulation of the same muscle at a time when no repetitive muscle action potentials were shown by the electrical records. These observations on mechanical responses of DCA-treated muscles to direct stimulation are regarded as evidence that repetition may occur after complete neuromuscular block of these muscles. If the assumption that repetition occurs in completely curarized muscle is plausible, then it may be concluded that treatment with DCA and a low K diet induces changes in the responsiveness of the muscle fiber itself.

To explain the disappearance of repetitive muscle spikes before the disappearance of the initial large spike in response to single indirect stimuli, during the progress of curarization, it is necessary to assume either that curare completely blocks all of the muscle fibers involved in the repetition or that it blocks repetition before it blocks single muscle responses. There is no experimental evidence for the

former assumption but observations on the effect of curare on the endplate potential offer support for the latter assumption. It has been shown that curare gradually reduces the height of the endplate potential to a level where neuromuscular transmission ceases (Kuffler, 1942). Eccles, Katz and Kuffler (1941) have shown that subparalytic doses of curare increase the initial rate of decay of endplate potentials in the cat's soleus. It is possible that curare action may reduce the potential change resulting from a single nerve impulse at the endplate region to an amount which just allows, at the peak of the potential change, the transmission of a single impulse through the muscle fiber. During the refractory period induced by the single response the potential may return sufficiently toward zero that it is no longer able to set up a repetitive impulse in the muscle fiber.

The repetition resulting from eserine treatment which is known to be abolished by curare (Brown, Dale and Feldberg, 1936) was much shorter in duration (fig. 4, 1C) than that seen in DCA-treated animals. Furthermore, the prolongation of repetition produced by single shocks in muscle treated with veratrine resembled the duration observed in DCA-treated rats. Bacq and Brown (1937) have shown that the effect of veratrine on the mechanical response of muscle to single stimuli is unaltered by complete curarization. These experimental facts regarding the nature of repetition in eserinized and veratrinized muscle add support to the view that DCA treatment induces changes in responsiveness of the muscle fiber substance.

Granting that repetition may be obtained by direct stimulation and that DCA treatment alters the response of the whole muscle fiber does not exclude the possibility that changes at the endplate region of the muscle fibers occur as a result of treatment with DCA and a low K diet. With the thought in mind that DCA treatment may produce a prolonged negative potential and a "slow wave" similar to those observed by Eccles, Katz and Kuffler (1941) in the eserinized cat's soleus, endplate potentials of the completely curarized soleus of the rat were observed. Only a slight prolongation of the endplate potential and no "slow wave" were found in the DCA-treated muscle (fig. 1, 1G). However, the failure of markedly prolonged negative potentials to appear in curarized muscle does not prove that such potentials are absent in the uncurarized muscle for according to Eccles, Katz and Kuffler (1942) the prolonged endplate negativity observed in the eserinized cat's soleus is greatly diminished by curare. These investigators also reported (1941) that subparalytic doses of curarine which had little effect on the endplate potential greatly reduced or abolished the "slow wave."

On the basis of the amount of developed tension and the size of action potentials of the after discharges it may be concluded that the repetition in DCA-treated animals involves a smaller portion of the muscle fibers than the repetition induced by eserine or veratrine (fig. 4). The somewhat regular rhythm frequently seen during repetition (fig. 1, 2A and 2E) suggests that muscle units are repeating several times. However, it should be emphasized that no evidence of regular rhythm appeared in many of the repetitive after discharges. The abrupt diminution in size of repetitive action potentials without interruption of an established rhythm (fig. 1, 2E) offers good evidence that multiple units were firing off synchronously before the diminution occurred.

In a study of the rat Darrow and Miller (1942) found that either DCA treatment or a low K diet produces a significant reduction of K content in skeletal muscle and only slightly lowers the level of K in cardiac muscle. In a more recent report Darrow (1944) has shown that 4 days of treatment of the rat with a combination of a low K diet and DCA reduces the K content of cardiac muscle significantly. Therefore, it seems safe to assume that the simultaneous low K diet and DCA treatment employed in the present study brought about a decrease of the K content in skeletal muscle. The abolition of repetition by intraperitoneal injection of KCl suggests that the repetition was a result of K imbalance. There can be no doubt that K had reached the muscle in sufficient quantity to be effective because definite potentiation of muscle tension had occurred at the point of abolition of repetitive muscle spikes (fig. 3, 1F and 2F).

The state of muscle in DCA-treated rats is somewhat similar to that existing in human myotonia and in a particular strain of goats showing congenital myotonia. As in the myotonic goat (Brown and Harvey, 1939) the repetition is abolished by exercise but not by curare. Potassium initiates repetitive discharges in the myotonic goat (Brown and Harvey, 1939) and it exaggerates the myotonic state in myotonia congenita of man (Russell and Stedman, 1936). On the basis of the observations in the DCA-treated rat that intraperitoneal injection of KCl first increases and then abolishes repetition, it seems reasonable to suggest that K in small quantities either excites or increases the excitability of muscle fibers depleted of this ion and that K in larger quantities reduces muscle excitability. The finding by Cumings (1939) of a marked reduction in K content of muscle in certain cases of human myotonia is consistent with the observed similarities of responses in myotonic muscle and DCA-treated muscle. On the other hand, it is surprising that administration of DCA abolished repetition in the muscle of myotonic goats (Gammon, Harvey and Masland, 1941) in view of the finding in our experiments that the amount of repetition is increased in rat muscle by prolongation of DCA treatment.

SUMMARY

1. The gastrocnemius muscle of rats injected with DCA and fed a low-K diet shows repetitive action potentials for initial responses of rested muscle to single shocks; the repetitiveness disappears when single shocks are repeated at the rate of 1 every 2 seconds. The repetitive discharges do not arise at the point of stimulation of the nerve.

2. During indirect stimulation with single shocks repetition is abolished by the time curare reduces the peak tension of the mechanical response about 25 per cent. However, the similarity of mechanical responses elicited by single direct shocks in completely curarized muscles (triangles in fig. 2, D) to mechanical responses showing repetition as a result of single indirect shocks in the same muscle before curarization (triangles in fig. 2, C) led to the conclusion that direct stimulation induces repetition in DCA-treated muscle after complete curarization.

3. Intraperitoneal injection of KCl in the DCA-treated animals first increases the amount of repetition and finally abolishes repetitiveness. The typical po-

tentiating effect of K on muscle tension is in evidence at the point when repetitive discharges disappear.

4. The repetition in veratrinized muscle which is not abolished by curare is more similar to the repetition in DCA-treated muscle than the repetition in eserinized muscle which is abolished by curare.

5. Although the evidence indicates that responsiveness of the muscle fiber substance is modified by DCA treatment the possibility of changes at the end-plate region of the muscle fiber, as a result of DCA treatment, is not disproved.

6. Some similarities of the effects obtained by DCA treatment and of the conditions existing in myotonia are pointed out.

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THE EFFECT OF DIETARY FAT UPON GASTRIC EVACUATION IN NORMAL SUBJECTS

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The effect of fat as it occurs in normally constituted, mixed meals upon gastric evacuation has not been studied previously. This is primarily because available methods for measuring gastric emptying are poorly adapted to the use of such meals. Recently a new method for detecting changes in the volume of the gastric contents has been devised and tested (1). The method permits use of meals of any desired composition, and does not interfere with normal gastric activity. In the present study the gastric evacuation of mixed meals containing various amounts of lard or hydrogenated vegetable oil (Crisco) has been determined in normal subjects.

It has been previously shown that olive oil delays the gastric emptying of an Ewald or gruel meal in man (2, 3). It has also been shown that potatoes cooked to contain 37.5 per cent fat are more slowly emptied from the stomach than boiled potatoes (4). However, the level of fat in a mixed meal at which gastric motor inhibition appears, the consistency of such inhibition in given individuals, and the proportion of individuals that exhibit gastric inhibition attributable to the fat content of a meal are not known. It was the purpose of this study to answer these questions.

METHODS. Thirty subjects were given 1500-calorie meals after fasting for six hours. The meals contained 53, 77 and 120 grams total fat, of which 25, 50, and 80 grams, respectively, were added lard or hydrogenated vegetable oil. The menus and composition of the test meals at the three fat levels are given in table 1. All meals were prepared by the same dietitian from food obtained in case lots to ensure uniformity of composition.

Four hours after each test meal, subjects were given 100 cc. of 20 per cent barium sulphate suspension. An upright, A-P film was immediately made. From changes in the area of the projected x-ray shadow of the stomach when the fat content of the test meal was varied, changes in the volume of the 4-hour gastric contents were detected. Each subject served as his own control. This procedure has been shown to be valid and reliable (1).

Test meals were given twice weekly. Two tests were conducted at each level of each type of fat. The order of the various test meals was rotated among the subjects. All data were treated statistically by the method of analysis of variance (5).

RESULTS. The area of the gastric shadow four hours following 1500-calorie meals containing three levels of lard and hydrogenated vegetable oil was mea-

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sured. The results of two tests at each fat level in 30 subjects are summarized in table 2.

TABLE 1

A. Menus of x-ray test meals; weight in grams

	A 53 GRAM FAT LEVEL	B 77 GRAM FAT LEVEL	C 120 GRAM FAT LEVEL
Fruit juice	100	140	
Chicken	100	90	85
Peas	50	50	50
Bread	30	40	40
Added fat (lard or HVO)	6		18
Preserves	30		
Sugar	100		
Pineapple	120	100	125
Cream sauce	100	100	90
Added fat (lard or HVO)	20	20	20
Chocolate sauce		50	100
Added fat (lard or HVO)		30	40
Ice cream	100	180	200
Water taken with meal (not included in analyses below)	400	400	400

B. Composition of x-ray test meals

	A	B	C
Total calories (calculated)	1475	1375	1500
Wet volume, cc	710	750	750
Wet weight, grams	850	920	835
Dry weight, grams	240	280	320
Added fat, grams (lard or HVO)	25	50	80
Total fat by analysis, grams	53	77	120
% fat of wet weight	6	8	14
% fat of dry weight	22	28	38

TABLE 2

Summary of effect of fat on gastric evacuation

TOTAL FAT CONTENT OF MEAL	LARD OR HVO INCLUDED IN MEAL	MEAN GASTRIC SHADOW AREA		MEAN AREA OF BOTH FATS
		HVO	Lard	
gms.	*gms.*	*sq. cm.*	*sq. cm.*	*sq. cm.*
53	25	165	156	160
77	50	177	174	175
120	80	196	195	195

Difference between means necessary for significance = 14.3 sq. cm.*

* Derivation of this term is given in table 3.

Since significant differences in gastric shadow area occurred between the three levels of fat, but not between types of fat at any level, it is evident that (a) in-

creasing the fat content of the test meal delayed the 4-hour gastric evacuation, and (b) lard and hydrogenated vegetable oil did not differ in their effect on gastric emptying.

The mean individual gastric shadow areas for repeated tests at each level of HVO and lard are given in table 3, together with an analysis of the data. Since it has been shown previously that repeated tests with a meal of constant composition do not differ significantly (1), single test results are omitted.

The analysis shows that significant differences in gastric shadow area occurred between subjects. From data in table 2 the intraclass correlation coefficient may be calculated as

$$\frac{2762}{2762 + 406} = 0.87.$$

This figure may be used as a measure of reliability for the study. The analysis further shows (a) highly significant differences in gastric shadow area between levels of fat, and (b) no significant differences between types of fat.

Discussion. It has been shown that the amount of fat incorporated in a mixed meal alters gastric evacuation. The total fat content of the test meals used in this study ranges from 6.0 to 14.0 per cent of their wet weight, or 22 to 38 per cent of their dry weight (table 1). This fat concentration includes the limits of ordinary dietary fat intake. No disagreeable symptoms followed any of the test meals. It is therefore apparent that the amount of fat that may be contained in the ordinary diet of man is capable of affecting the emptying of the stomach, just as pure fats have been shown to delay gastric evacuation of an artificial meal (2, 3).

The delay in gastric evacuation that occurred when the fat content of a meal was increased was a consistent characteristic for given individuals, and was not dependent upon uncontrolled factors apart from the composition of the test meal. This is evident from the following considerations: (a) there are significant differences between individuals in gastric shadow area, and (b) these individual differences are consistent as measured by the high reliability coefficient of 0.87.

Inspection of table 3 shows that of the 30 subjects, five showed no delay in gastric evacuation between the 53 and 77 gram fat-levels (subjects 5, 6, 14, 20, and 29). Of these five subjects, three showed no delay in 4-hour gastric emptying when the dietary fat was increased to 120 grams (subjects 5, 20, and 29). This does not necessarily mean that some normal persons lack a gastric inhibitory mechanism for fat, but only that error in the technique may have hidden the gastric response to fat in three of the subjects. If one assumes that the 30 subjects in this study are a random sample of the normal population, it may be concluded with 95 per cent probability that from 70 to 98 per cent of the normal population may be expected to show gastric inhibition attributable to the fat content of meals within the range and using the method of this study (95 per cent confidence limits for a sample size of 30).

No attempt has been made to measure quantitatively the degree of gastric retention four hours after a test meal; such is beyond the limitations of the method of study.

TABLE 3

Effect of fat on gastric evacuation

Means of repeated tests at three levels of HVO and lard.

TOTAL FAT..........	53 GRAMS		77 GRAMS		120 GRAMS	
Added fat..........	25 grams		50 grams		80 grams	
Subject	HVO	Lard	HVO	Lard	HVO	Lard
1	209	222	225	215	251	249
2	157	134	177	177	175	177
3	166	177	183	186	215	240
4	213	167	219	217	223	239
5	188	189	178	182	189	199
6	202	202	196	192	216	221
7	162	196	183	183	202	210
8	125	132	163	138	156	151
9	154	109	169	162	199	204
10	161	161	174	152	182	179
11	157	117	164	156	195	181
12	162	131	168	164	180	193
13	159	166	171	159	189	199
14	120	124	118	114	131	119
15	137	130	169	134	184	187
16	181	107	154	138	175	173
17	198	154	224	200	269	284
18	136	151	170	152	191	195
19	182	166	183	186	207	215
20	210	199	202	206	207	208
21	172	173	165	201	213	191
22	139	136	161	195	182	194
23	168	159	181	187	193	195
24	155	156	192	188	193	205
25	163	178	168	203	195	199
26	118	112	122	146	165	150
27	150	142	157	149	186	156
28	148	160	161	171	194	174
29	209	180	218	186	204	191
30	159	155	182	168	203	173
Average.....	165	156	177	174	196	195

Analysis of variance; F° 0.01 = 3.13

SOURCE OF VARIATION	DEGREES FREEDOM	MEAN SQUARE	R F RATIO	SIGNIFICANCE
Differences between subjects..........	29	2762	7	high
Differences between fat levels........	2	15166	37	high
Differences between fat types........	1	836	2	none
Discrepancy (interaction plus error)..	147	406		

St. error of differences between means $= \sqrt{406} \times \sqrt{\frac{1}{30} + \frac{1}{30}} = 5.20.$

Difference in means necessary for significance $= 5.20 \times 2.75^{*} = 14.3$ sq. cm.

* 0.01 value of t.

SUMMARY AND CONCLUSIONS

1. Thirty normal subjects were given 1500-calorie test-meals of constant weight and volume and containing 53, 77, and 120 grams total fat. Of this, 25, 50, and 80 grams, respectively, were lard or hydrogenated vegetable oil (Crisco). Four hours after the test meal the stomach was outlined with a small amount of barium sulphate suspension and an x-ray film was immediately made. Changes in the 4-hour volume of the stomach were detected by measuring the area of the projected gastric shadow.

2. Delayed gastric evacuation attributable to the fat content of the test meal was statistically significant. Twenty-five subjects showed a delay at four hours when the fat content by wet weight was increased from 6 to 8 per cent and 27 subjects showed delayed gastric evacuation when the fat was increased from 8 to 14 per cent.

3. No significant differences were found between lard and hydrogenated vegetable oil in their effect on gastric emptying. No gastro-intestinal symptoms followed any test meals with either lard or hydrogenated vegetable oil.

4. The gastric inhibition which occurred when the fat content of the meal was increased was a consistent characteristic for given individuals.

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SYSTEMIC BLOOD PRESSURE AS A FACTOR IN THE ABSORPTION OF SALINE FROM THE SMALL INTESTINE

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Gellhorn and Northup (1) found that the rate of absorption of glucose from isotonic solutions in the gut of the frog may or may not be increased by an elevation in the perfusion pressure of Ringer's solution entering the mesenteric artery. They suggested that the instances in which increased rate of absorption was found were due to enlargements of the area through which diffusion might take place, by increases in the extent of the active capillary bed.

Wells (6) found that the rate of absorption of isotonic saline from isolated loops of the small intestine of the dog could be decreased by producing congestion of the mesenteric veins. He explained his results on the basis that the force of absorption in the case of isotonic solutions is the colloid osmotic pressure of the blood plasma, and that any factor which decreases or counteracts this, such as increased filtration pressure, reduces the rate of absorption.

Diffusion and osmosis, however, are not the only forces active in absorption. This has been most clearly demonstrated by Visscher *et al.* (5) in experiments on the movements of water and ions, revealed by the presence of isotopic tracers, between intestinal loops and blood in dogs. Some other force is apparently present which causes a functional circuit of fluid between intestinal lumen and blood which brings about a net movement of ions and water into the blood when absorption is occurring.

The determination of the rôle played by blood pressure upon the rate of absorption can furnish evidence, indirect though it may be, which may have some bearing on the current hypotheses of the forces of absorption. In addition it will show how important a variable the level of blood pressure is when the influence of other factors upon absorption rate is being assessed. The presence in the population of a considerable number of individuals with elevated blood pressure makes the determination of some practical importance as well.

Methods. Dogs, weighing between 4.6 and 27.2 kgm., were anesthetized with 300 mgm./kgm. of sodium barbital intravenously after preliminary induction with ether. The left common carotid artery was ligated and a lucite-covered electrode inserted into the artery distal to the ligature so that the exposed tip lay in the carotid sinus region. The other electrode was wrapped around the artery, and both were connected to the secondary of a Harvard inductorium supplied with a 7 volt current and arranged to produce tetanic stimuli. Blood pressure was recorded from the femoral artery with a mercury manometer.

The abdominal cavity was opened by a midline incision, and a Moreau loop, 1 m. in length, made of the terminal end of the small intestine. The loop was

washed with warm isotonic glucose and the wash fluid forced out by gently stripping. The loop was returned to the abdominal cavity, and the incision closed with clamps. After blood pressure equilibrium was attained, the carotid sinus region was stimulated (with the secondary coil set between 3 and 6 cm. from the primary) in order to produce a fall in blood pressure. If the fall was well marked and sustained, 100 cc. of isotonic saline was introduced into the Moreau loop. If otherwise, stimulation was stopped, and upon attainment of blood pressure equilibrium again, saline was introduced.

At the end of 30 minutes in both types of experiment, the amount of fluid left in the gut was measured in a graduate and chloride determined by the Van Slyke modification of the Volhard method. The average blood pressure during the absorption period was determined with a planimeter.

The above routine was altered in some cases. In 4 experiments the carotid-sinus region was denervated in an attempt to elevate the blood pressure and in one of these the vagi were sectioned in addition. In 2 experiments the carotid artery was not ligated, nor were the electrodes put in place. In a few additional cases the saline was placed into the intestine without any stimuli being applied to the sinus region either before or during the absorption period.

Results. The absorption of fluid and of chloride was determined in 47 dogs in which the carotid sinus region was stimulated and in 38 in which it was not. The results are shown in table 1. A comparison of the absorption rate in stimulated with that in non-stimulated dogs where the ranges of blood pressure overlapped and where, consequently, the effect of the level of blood pressure was controlled, revealed (4) a detrimental effect of the stimulation on absorption. This makes it necessary to separate the results into two series: one in which stimulation was present, and one in which it was not.

In the stimulated dogs, blood pressures, with but two exceptions, ranged from 71 to 137 with a mean of 101 mm. Hg. Of the 100 cc. of 0.9 per cent NaCl introduced, an average of 43.4 per cent of the fluid and 51.7 per cent of the chloride had been absorbed during 30 minutes. There was no apparent relation between blood pressure level and absorption rate within this group, nor was there any relation between absorption rate and the amount of blood pressure reduction from the control level prior to stimulation.

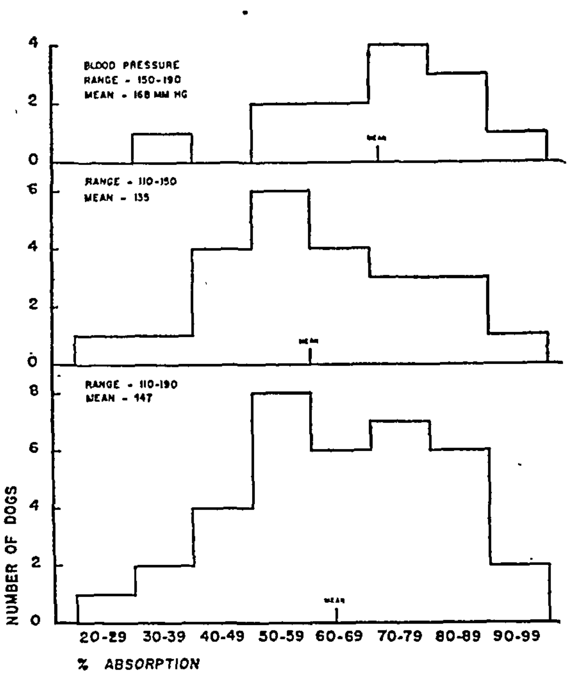
In the non-stimulated dogs, blood pressures ranged from 103 to 189 with a mean of 144 mm. Hg. The distribution of the percentage absorption of fluid among the 36 dogs having blood pressures between 110 and 190 is shown in figure 1. Likewise is shown the distribution when the group is subdivided into groups having blood pressures between 110 and 150 and between 150 and 190 mm. Hg. The curve for the 110-190 group shows two peaks suggesting the presence of individuals from two differing populations. The removal of the 13 experiments in the 150–190 range improves the distribution considerably.

The distributions in respect to chloride absorption for the same groups of non-stimulated dogs are shown in figure 2. Since the values are not so widely scattered as in the case of fluid absorption, the presence of two differing populations is not so obvious on simple inspection.

TABLE 1

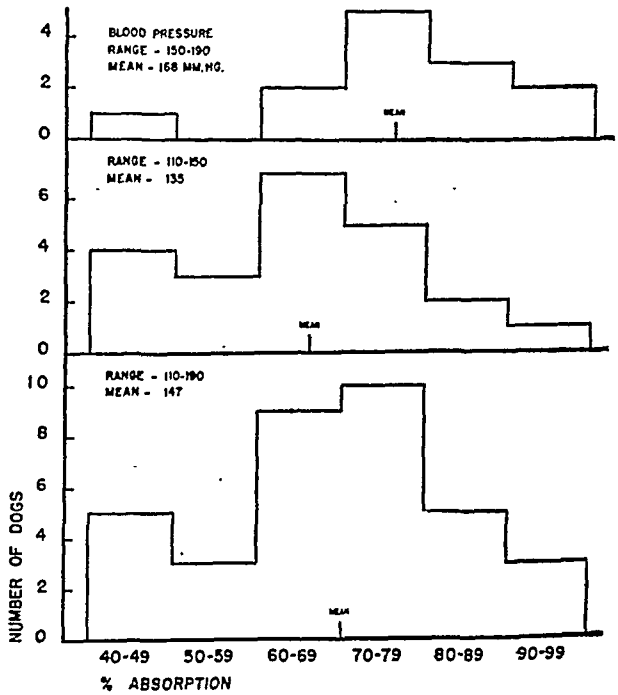
Blood pressure and absorption from 100 cc. 0.9 per cent NaCl in the small intestine of the dog during 30 min.

NUMBER OF DOGS	BLOOD PRESSURE RANGE	AVERAGE BLOOD PRESSURE	AVERAGE FLUID ABSORPTION	AVERAGE CHLORIDE ABSORPTION
Carotid sinus region stimulated				
	mm. Hg	*mm. Hg*	%	%
16	50–90	80	44.5	52.7
16	90–110	100	42.3	49.8
15	110–160	124	42.0	52.7
47	50–160	101	43.4	51.7
Carotid sinus region not stimulated				
10	100–130	119	57.0	63.7
23	110–150	135	60.0	66.0
13	150–190	168	71.5	75.9
38	100–190	144	62.7	68.9



DISTRIBUTION OF FLUID ABSORPTION IN DOGS (BARBITALIZED)

Fig. 1



DISTRIBUTION OF CHLORIDE ABSORPTION IN DOGS (BARBITALIZED)

Fig. 2

When the average absorption rates for fluid and for chloride of the non-stimulated dogs (lower part of table 1) with blood pressures in the 110–150 range are compared with those in the 150–190 range it is seen that blood pressure exerts a noticeable effect. The average values for fluid absorption in the

two ranges were 60.0 and 71.5 per cent; and for chloride, 66.0 and 75.9 per cent respectively. The differences are 11.5 for fluid and 9.9 for chloride. These are statistically significant at the 0.05 and 0.04 levels of probability respectively. The effect is demonstrated more convincingly when a comparison is made of the average absorption rates in dogs having blood pressures in the 100–130 range with those in the 150–190 range. Here, the differences for fluid and chloride with their levels of probability are 14.5 at the 0.04 level and 12.2 at the 0.03 level respectively. If the 4 experiments in which the carotid sinuses were denervated are omitted the differences between the groups become greater and the levels of significance are reduced to 0.02 and below. These results show that the absorption rate is greater for isotonic saline at higher than at lower blood pressures.

Discussion. Although the saline solutions used in these experiments were approximately isotonic with dog plasma, a diffusion gradient favoring the entrance of chloride into the blood must have been present, at least initially, since the concentration of chloride as NaCl in dog plasma is only about two-thirds that of isotonic saline. It is questionable whether any close comparison should be made between our results and those of Gellhorn and Northup (1) in which essentially surviving loops of gut were used, 'blood pressure' was artificially produced and an infinitely steep diffusion gradient was maintained with a different substance (glucose) in a vastly different species (frog). However, their finding that local circulatory changes can modify the results when perfusion pressure is raised, should not be ignored. Although it is frequently assumed, it is not known whether there is an active adaptation in the local circulation of the gut when absorption is taking place, nor is it known how that local circulation is affected when systemic blood pressure is high as it was in the 150–190 mm. Hg group of dogs reported here. It is possible that the beneficial effect of high blood pressure on absorption in these experiments was due to a widening of the active capillary bed and the consequent enhancement of transport by diffusion.

If the effective colloid osmotic pressure of the blood plasma be considered as a predominant factor in absorption, as suggested by Wells (6), then high systemic blood pressure would be expected to favor capillary filtration and intestinal secretion rather than absorption unless rather drastic compensatory changes in vasomotion in the gut circulation were simultaneously made. The greater absorption rate in our experiments when blood pressure was high obviously does not support this view.

The main force of absorption may be due to the activity of specific absorbing cells in the wall of the intestine. Recent studies (5) indicate that physicochemical work must be performed by some means to cause movements of water and ions against normal osmotic forces and against diffusion gradients. The site of such activity is unknown, but it may be supposed to lie somewhere in the region between the free border of the epithelial layer and the capillaries of the intestinal mucosa. When absorption is occurring, according to the fluid circuit concept, the concentration of the solute must be increased on the capillary side of the active region. Since diffusion factors, although not predominant,

are known to affect absorption, any mechanism which would minimize the building up of a local concentration of absorbed solute at the border of the active region should favor the more rapid absorption.

We have found absorption to be favored in the presence of high systemic blood pressure. Under these circumstances, the condition of the circulation through the gut must depend on the degree to which its blood vessels are active or passive in the production of the high pressure. Herrick *et al.* (2) have shown that blood flow in the mesenteric artery may be increased during the blood pressure rise accompanying exercise in spite of concomitant vasoconstriction in the viscera. Greater than usual pressure in the arteriolar end of the capillary network would produce a faster formation of tissue fluid (3), which, if conditions at the venous end were favorable, would be balanced by faster absorption of tissue fluid, or in the absence of the latter, faster formation of lymph in the lymphatics. On the other hand, if the blood vessels of the gut were relatively passive, one would expect the capillary bed to be wide in extent due to the presence of many active capillaries. All of these factors may act to cause a more rapid renewal or circulation of the extracellular fluid in the active region of the mucosa and thus effect an unusually brisk removal of solute from this region. If this explanation is correct, the absorption of water must be closely associated with that of solute, because fluid absorption was also affected by the blood pressure level. A more precise definition of systemic blood pressure as a factor in absorption awaits the better understanding of the local conditions, both circulatory and absorptive, in the intestinal mucosa.

SUMMARY AND CONCLUSIONS

In 47 barbitalized dogs, in which the carotid sinus region was electrically stimulated to produce a fall in blood pressure, there was no relation between the rate of absorption of saline from Moreau loops of the small intestine and the blood pressure level throughout the range: 71 to 137 mm. Hg.

In 38 dogs, not stimulated, the rate of absorption of both fluid and chloride was statistically greater when blood pressure ranged from 150 to 190 than between either 100 and 130 or 110 and 150 mm. Hg.

It is suggested that the beneficial effect of high systemic blood pressure is due to better removal of absorbed solute in the gut mucosa.

The technical assistance of R. E. Richard is gratefully acknowledged.

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PHYSIOLOGICAL EFFECTS OF A PLASMA PROTEIN: BLOOD PRESSURE, LEUCOCYTE CONCENTRATION, SMOOTH AND CARDIAC MUSCLE ACTIVITY[1]

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It is becoming increasingly evident that plasma may normally possess unrecognized proteins capable of producing physiological responses. However, in order to study such proteins they must first be obtained in suitable concentration. We have had an opportunity to examine a new plasma fraction recently described (1). Intravenous administration has revealed a powerful depressor substance which is non-dialyzable and relatively heat stable. The depressor substance is insoluble in water, but soluble in saline. Its activity is not destroyed by reduction with cysteine or glutathione. It is precipitated from plasma within the limits 20 to 29 per cent of saturation with ammonium sulfate. In addition this fraction has been found to possess a leucopenia producing effect, and a stimulating effect on smooth muscle. It inhibits the activity of the turtle heart.

General methods. *Biological materials.* Dogs were used for studying depressor and leucopenia producing effects of plasma fractions. They were of either sex and averaged about 8 kgm. body weight. Approximately 100 were used in this study. Generally they were given nembutal anesthesia, but in a few instances morphine plus urethane was used. Blood pressure measurements were made with a mercury manometer by cannulating the carotid artery. Both right and left femoral arteries and veins were exposed. The left femoral artery was cannulated for the purpose of obtaining blood samples.

Guinea pigs were obtained from regular stock. They were given morphine plus urethane anesthesia. Blood pressure measurements were made as with the dogs.

Smooth muscle was obtained from the duodenum of the rabbit. Tyrode solution was used to bathe the tissue and the ordinary arrangements for recording movements were used.

Turtles were of such size as to have a plastron 8 inches long. For perfusion experiments the right innominate artery and left axillary vein were cannulated. The other vessels were ligated. Ringer-Locke solution was used as perfusion fluid. The venous pressure was arranged so as to give maximum output of the heart against a low arterial pressure.

General blood studies. Whole blood clotting times were done by the Lee-White method. Sedimentation rates were measured in Cutler tubes. Seven parts of blood were mixed with 1 volume of 1.85 per cent potassium oxalate. Red and

[1] Aided by a grant from the National Institute of Health.

white cell counts were made on similarly oxalated blood by conventional methods. The same person performed all counts including differentials.

Plasma fraction. These preparations were made as described by Loomis, George and Ryder (1). This plasma fraction is very potent in fibrinolysin activity, and our first purpose in working with the product was to study the *in vivo* effects of fibrinolysin. The plasma fraction containing fibrinolysin, along with other proteins, will be referred to as fibrinolysin.

EXPERIMENTAL RESULTS. *Dogs.* The fibrinolysin, in saline solution, was injected into the femoral vein. The mean blood pressure in dogs which received 100 to 200 mgm. fibrinolysin (total of 50–400 units) per kgm. of body weight fell 50

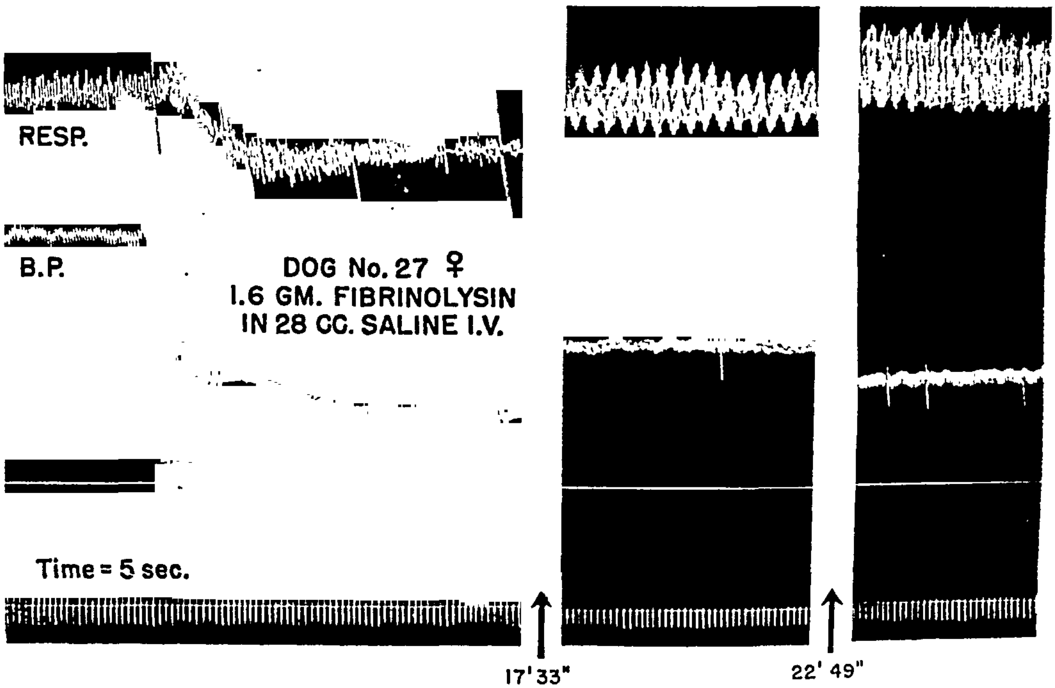

Fig. 1. Effect of injecting 1.6 grams of fibrinolysin. The dog weighed 8 kgm., and the initial blood pressure was 134 mm. Hg.

per cent or more within 15 seconds after the start of administration (fig. 1). In the experiment shown on figure 1 the initial depression in blood pressure lasted about 12 minutes, then there was partial recovery followed by a secondary fall. The pressure then remained low 4 hours and 52 minutes at which time the experiment was terminated. In about 5 per cent of the animals death occurred before an arbitrary termination of the experiment. Generally one finds that the gingival line becomes pale. The spleen is contracted and the intestinal area is congested with blood. With 10-20 mgm. per kgm. of body weight the initial fall is similar to that with large doses but recovery is rapid.

Figure 2 gives a more detailed analysis of the events immediately following intravenous administration. The record shows the results for blood pressure, respiratory rate and heart rate following the administration of 2.8 grams of

fibrinolysin to a 6.8 kgm. dog. The heart rate increases as the blood pressure falls. Therefore, the response of the heart may be reflex in origin. Although the blood pressure remains low the heart rate rapidly returns to the pre-injection rate. Respiration also increases and the time relationships are such that this effect also may be due to reflex activity.

Guinea pigs. As in the dog experiments the intravenous administration of the fibrinolysin resulted in a profound fall in blood pressure (table 1). In this species both the primary and secondary falls occurred. The responses are altogether similar to those in the dogs.

General observations. It seemed necessary to minimize the possibility that the fall in blood pressure might be due to histamine. For that purpose benadryl (β-dimethylamino-ethyl benzhydryl ether hydrochloride) was used, because this drug has been shown to neutralize the effect of histamine (2). Three to 6 mgm. per kgm. of body weight were given intravenously from 2 to 5 minutes prior to the fibrinolysin. The blood pressure fall was not prevented by benadryl, and we consider this fact as strong evidence that histamine is not directly involved in the fall in blood pressure.

In a similar manner atropine (1 mgm./kgm. body wt.) was injected from 2 to 5 minutes prior to the fibrinolysin. This also failed to modify the blood pressure fall. This is interpreted to mean that acetylcholine and the parasympathetic system are not essential factors in this response.

Clotting times following the intravenous administration of fibrinolysin were not significantly altered from the pre-injection control values. Hematocrit measurements and sedimentation rates measured at intervals following administration of the fibrinolysin also showed no significant deviations from the control measurements.

Leucopenia. A profound leucopenia developed within 3 minutes following the intravenous administration of fibrinolysin in dogs and it lasted from 30 minutes to 4 hours (fig. 3). Erythrocyte counts made on blood taken from the femoral artery and femoral vein remained unaltered. In the majority of cases the differential pattern showed unequivocally that the polymorphonuclear leucocytes practically disappeared. The lymphocytes decreased only slightly in number. The net result then was the predominance of lymphocytes in a blood smear. Although the leucopenia usually persisted from 2 to 4 hours, the differential pattern in most cases returned to the normal ratio in about 45 minutes following the injection. During the recovery period a 5 to 15 per cent increase in young cells (band forms) frequently occurred.

In vitro experiments showed that the leucocytes are not destroyed directly by fibrinolysin.

In order to follow the cellular changes over a longer period of time fibrinolysin was given by vein to an unanesthetized dog and cell counts were made at intervals for several days thereafter. The results are shown in figure 4. The total white cell count dropped as it does when fibrinolysin is given with anesthesia. The lymphocytes were affected less than the neutrophiles thus giving the same pattern which has been observed repeatedly. The principal effect lasted 3 hours.

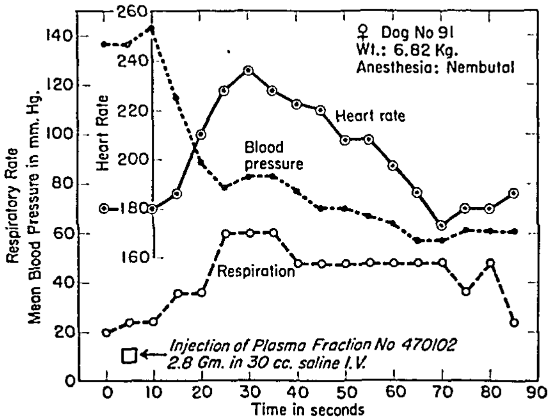

Fig. 2

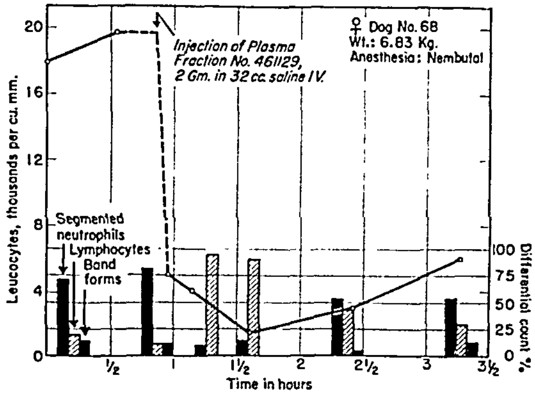

Fig. 3

Fig. 2. Detailed analysis of changes in heart rate, respiration and blood pressure immediately following the intravenous administration of fibrinolysin.

Fig. 3. Effect of fibrinolysin on léucocytes.

TABLE 1

Effect of intravenous administration of fibrinolysin in guinea pigs

GUINEA PIG NO.	FIBRINOLYSIN, MGM./KGM. BODY WT.	TIME TO INJECT	INITIAL MEAN BLOOD PRESSURE	GREATEST REDUCTION IN MEAN BLOOD PRESSURE	TIME TO RETURN TO NORMAL	COMMENTS
		sec.	*mm. Hg*	*mm. Hg*	*min.*	
1	300	45	70	70		Animal died in shock
2	300	130	70	20	8.3	Complete recovery
	17 min. after 1st injection: 100	55	80	20	3.8	Complete recovery
	33 min. after 2nd injection: 300	35	68	68		Animal died in shock
3	400	25	60	40	14.5	Large change in character of blood pressure record, but complete recovery
	75 min. after 1st injection: 400	30	80	64	20	Complete recovery
4	470	45	80	56	15	Good recovery
	20 min. after 1st injection: 100	25	90	24	5	Complete recovery
5	500	43	58	34		
	5 min. after 1st injection: 100	18	42	20	4.5	Back to blood pressure level prior to 1st injection in 10 min.
6	500	37	74	44		Injection before blood pressure recovery
	1.8 min. after 1st injection: 123	12	30	4		3rd injection before blood pressure recovery
	2.2 min. after 2nd injection: 568	22	26	26		Animal died in shock

On the second day there was a definite leucocytosis involving especially the neutrophiles. By the third day the white cell and differential count had returned to normal. It is clear from our analysis that, with the return of the white cells, the band forms do *not* predominate. Perhaps the original cells return.

A new plasma factor? Thus far the question has not been considered whether the substance producing these physiological responses is fibrinolysin or some other substance in the preparation. Fibrinolysin, as prepared (1), is known to contain several protein components. Our first experiments, planned to test the question just raised, definitely indicated that something other than fibrinolysin is acutally involved. Fibrinolysin is known to be destroyed by an inhibitor present in plasma (3, 4). This inhibitor destroys its capacity to lyse fibrinogen

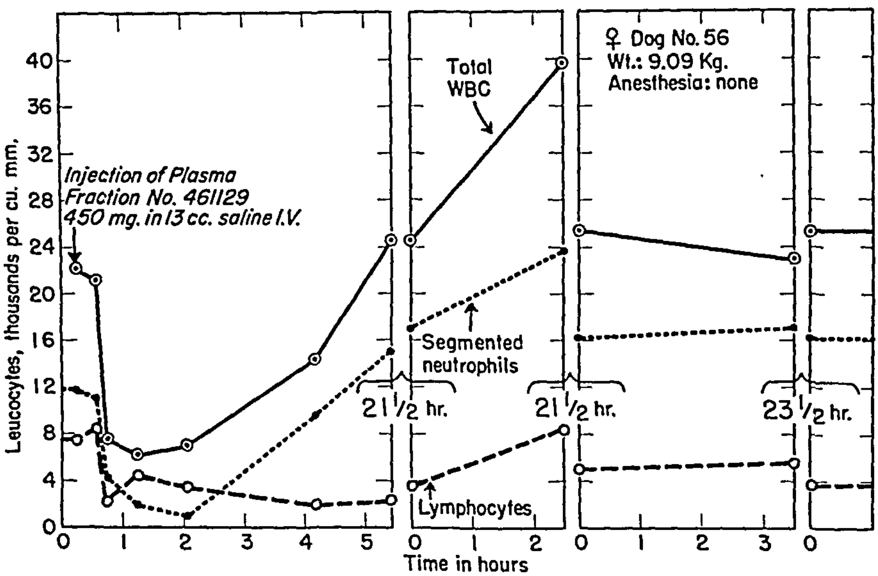

Fig. 4. Effect of fibrinolysin on leucocyte count of an unanesthetized dog.

and fibrin. It also destroys its capacity to inactivate prothrombin. We therefore mixed 1 gram of fibrinolysin with 50 cc. of oxalated dog plasma. After 1 hour of standing at room temperature practically all fibrinolysin activity had been destroyed. Upon intravenous injection of this mixture the depressor effect was obtained as usual. This experiment was repeated several times with the same results. It is clear that the depressor effect was not associated with the reactive groups responsible for fibrinolysis and an entirely different molecule probably produced the effect.

Next a search was made for a chemical means to destroy fibrinolysin. It was found that cysteine and glutathione completely destroys fibrinolytic activity. Solutions of fibrinolysin, treated with 1 per cent cysteine or glutathione, and possessing no fibrinolysin, as shown by actual quantitative tests, still produced the depressor effect.

Since a new substance is under consideration we have chosen to refer to it by the name *vascularin*.

Attempts were made to further purify vascularin. An important aid to that end was the observation that it is more resistant to heat than most plasma proteins. About 8 grams of fibrinolysin, prepared as described by Loomis, George and Ryder (1) were dissolved in 0.9 per cent sodium chloride, placed in a 500 cc. Erlenmeyer flask, and rapidly heated to 90°C. in a boiling water bath. The flask was rotated rapidly in order to facilitate heating. At 90° a change was made immediately to ice cold water so that quick cooling resulted. This heating coagulates about 80–90 per cent of the protein, but not the vascularin. Apparently not much vascularin activity is lost; however, our quantitative data on the latter point are not very exact. The coagulated protein was then removed by cen-

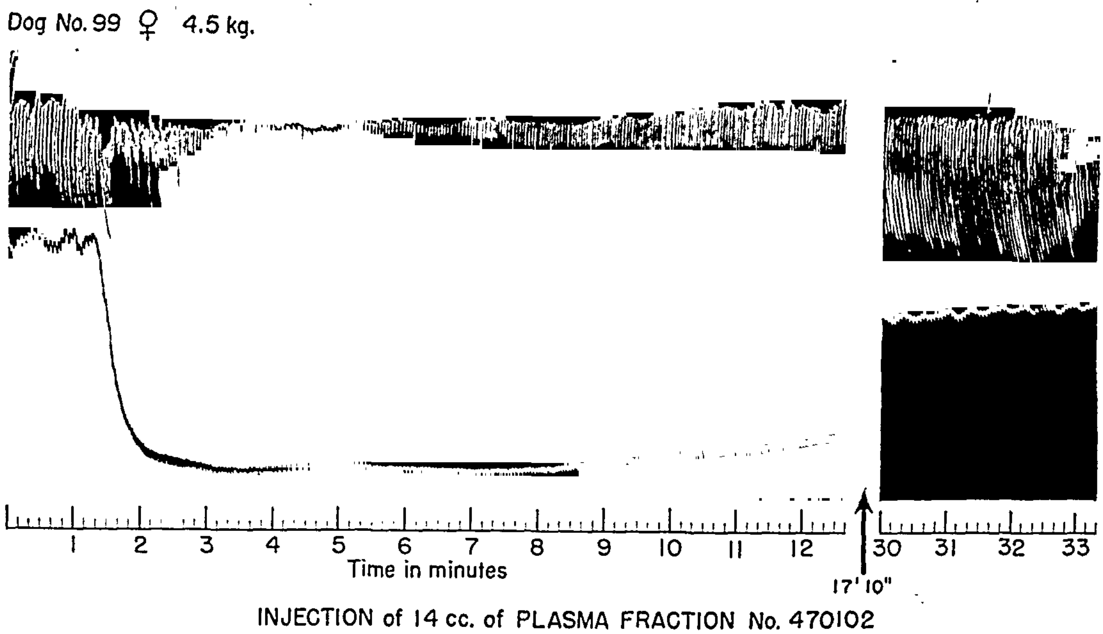

Fig. 5. Effect of intravenous administration of vascularin purified as described in text. The initial blood pressure was 150 mm. Hg.

trifugation and the supernatant fluid was shell frozen and dried from the frozen state. The dry material was dissolved in a small amount of saline and dialyzed against saline to obtain physiological concentrations of salt.

Figure 5 shows the depressor effect of this preparation when injected into the femoral vein of a 4.5 kgm. dog. This kind of preparation also produces leucopenia.

Smooth muscle. Gross observation of the gut following the administration of vascularin revealed marked activity. To test the possible direct effect of the protein on isolated intestinal muscle strips, a short segment of rabbit gut was immersed in Tyrode solution at 37°C. and the contractions of the longitudinal muscle were recorded. The vascularin to be tested was prepared from 1 gram of fibrinolysin by the method described above. It was dissolved in 30 cc. Tyrode solution and throughly dialyzed against Tyrode solution. The activity of the muscle was then tested in this solution. Within 1 to 5 minutes the tonus of the muscle had markedly increased and the amplitude of the contractions became

much greater than during the previous control period (fig. 6). Atropine decreased the marked tonus of the muscle, but the amplitude of the contractions was not affected. Vascularin invariably produces intense activity of the isolated rabbit gut.

Perfusion experiment. Perfusion experiments with the isolated turtle heart were performed as described above. When vascularin was introduced into the inflow tube on the venous side the cardiac output fell immediately to zero. Visible contractions usually continued at the normal rate but occasionally the heart would stop in diastole. When the vascularin was washed away the output returned rapidly to normal even if the heart action had ceased.

Preliminary purification of vascularin. At the slaughter house bovine blood was mixed with anticoagulant (1.85 per cent $K_2C_2O_4 \cdot 2H_2O$ plus 0.5 per cent $H_2C_2O_4 \cdot 2H_2O$) in the proportion of 1 part anticoagulant to 20 parts of blood. Plasma was obtained by centrifugation. To remove fibrinogen, 5,000 units of commercial thrombin (Thrombin, Topical, Parke, Davis & Co.) were added to 4 liters of plasma. Saturated ammonium sulfate solution was then added to the defibrinated plasma to 20 per cent of saturation. After centrifugation the precipitate was discarded. The supernatant was then brought to 30 per cent of saturation. After standing over night in the refrigerator at 5°C., the supernatant fluid was decanted and the precipitate packed by centrifugation. The precipitate was dissolved in 0.9 per cent sodium chloride and dialyzed for several hours against running tap water. The solution, about 200 cc. in volume, was placed in an Erlenmeyer flask, and the flask rotated in a boiling water bath until the temperature of the solution reached 90°C. A large mass of coagulated protein was obtained. The flask was rapidly cooled in ice water and the coagulum separated by filtration. The filtrate was then mixed with about 25 cc. of packed magnesium hydroxide paste. The mixture was centrifuged and the supernatant fluid was decanted. This solution was then shell frozen and dried from the frozen state by the method of Seegers (5). The dried material was dissolved in saline and dialyzed against repeated change of saline before being used for physiological tests. This vascularin preparation contains tyrosine and has no fibrinolytic activity.

Repeated experiments with this material prepared from different bovine plasma collections over a period of time have resulted in almost identical effects on the blood pressure of dogs, the white cell count of dogs, and the smooth muscle of the rabbit gut.

Discussion. There is no method for measuring the concentration of this factor in plasma and, therefore, one is handicapped in this work. It might be suggested, for example, that vascularin is a denatured protein and is not normally present in plasma. A transitory leucopenia has been produced with 2 grams' dried plasma. This result and the fact that 2 grams' bovine globulin (50 per cent saturation with ammonium sulfate at 0°C.) produced a very marked, though transitory, leucopenia leads to the belief that this factor is present in plasma as such and is not the result of chemical reagents acting on plasma. Two grams' crystalline bovine albumin had no effect. If the careful handling of the crude globulin fraction activated a precursor this does not detract from its

possible physiological importance, because physiological activators are numerous and one could exist for the purpose of activating a precursor of the factor being discussed.

In the preliminary purification procedure described above 42 mgm. of protein were obtained from 4 liters of plasma. Since the original concentration in plasma is not known we cannot calculate the per cent yield. Furthermore, there is no quantitative information as to the purity of the product. It could be reasonably free of foreign proteins because only 3 mgm. have produced a definite transitory leucopenia in a dog. Likewise the per cent yield of the method described might be good, because many modifications of the description given above resulted in lower yields. Let us then make the reasonable assumption that the purity of the product was 20 per cent and the yield also 20 per cent. Then 4 liters of plasma would contain 42 mgm. vascularin, and it would represent about 0.015 per cent of the total plasma proteins. This would escape attention in routine electrophoretic analysis of plasma. Assume unreasonably a yield of only 1 per cent and a purity of only 1 per cent. Then it would represent 1.5 per cent of the total plasma proteins and could still escape detection in electrophoretic patterns of plasma. It is very likely, therefore, that vascularin is present in normal plasma in small concentration.

The presence of a depressor factor in the globulin fraction of human and horse serum has been mentioned (6, 7) but apparently no extensive study has been completed. The physiological effects of this substance in concentrated form are of such character as to make it necessary to give consideration to the question of its normal rôle in health and disease. Although it might be suggested that sensitization phenomena come to be involved we do not consider that possibility seriously because it is not in accord with the known facts.

Shock-like reactions in dogs have been described (8, 9) as following the injection of ascaris extract and hydatid fluid. After 1½ to 2 minutes the blood pressure dropped, the blood became incoagulable, and the sedimentation rate increased. With vascularin the fall in blood pressure is immediate, there is very little change in clotting time and no increase in sedimentation rate. In view of these differences the substances cannot be considered to be identical in their physiological actions.

The non-specificity of leucopenia in dogs is well known. Pyrogens have notably been known to produce a leucopenia (10, 11). However, in the above described experiments, it seems unlikely that pyrogens could have produced the effects because of the method of preparation of the plasma fraction. It is also of significance that pyrogens generally are water soluble (12) while the active principle obtained from bovine plasma, with which this paper is concerned, has been shown to be insoluble in water.

Transitory leucopenia has been produced following the intravenous administration of substances of large molecular weight. For example, glycogen, gum acacia and starch have been used (13). Inasmuch as the leucopenia was accompanied by extremely high sedimentation rates it was postulated that rouleaux formation caused the larger red cell masses to occupy a position in the axial stream thus forcing the white cells to the periphery where they tend to re-

main either because of slower fluid movement or adherence to the vessel walls. This is not the explanation of the leucopenia found in our experiments because we found normal sedimentation rates. In no case in our series was the sedimentation rate significantly increased.

Other workers have offered an explanation for the transitory leucopenia produced by the intravenous administration of glycogen (14). It has been explained that the leucocytes collect in the lung capillaries. Pulmonary tissue sections made from the dogs in our experiments show no evidence of concentration of leucocytes. In addition it is extremely unlikely that the protein fraction with which we are concerned contains glycogen or is in any way similar to glycogen in its action.

SUMMARY

A protein concentrate has been obtained from bovine plasma which has a profound depressor effect when injected intravenously, either in dogs or guinea pigs. The depressor effect is immediate in its onset. Depending upon the quantity administered, animals may or may not survive. We refer to this substance as vascularin. It also produces a leucopenia, which is sudden in onset, but reversible in character. The lymphocytes are relatively less affected than the granulocytes.

It stimulates the smooth muscle of the rabbit intestine. In perfusion experiments with the isolated turtle heart, there is a decrease in cardiac output, and these effects on the heart are reversible.

Vascularin has the properties of an euglobulin, it is non-dialyzable, it is unusually heat stable for a plasma protein, and it does not lose its physiological activity when reduced with cysteine or glutathione.

Glutathione and cysteine destroy the activity of fibrinolysin.

We wish to thank Eugene C. Loomis for supplying us with generous quantities of fibrinolysin. We also wish to thank Parke, Davis and Company for supplying funds for Research in Physiology.

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ADRENAL RESPONSE TO TOTAL BODY X-RADIATION

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Our interest in the adrenal response to total body x-radiation has been stimulated by several considerations. Among these may be stated 1, the similarity between the changes produced in blood and lymphoid tissue by x-ray and by single injections of either adrenal cortical extract or pituitary adrenotrophic hormone (2, 3); 2, the potentiating effect of urethane, which presumably stimulates the adrenals, on x-ray toxicity (6, 10, 11); 3, the similarity between many of the terminal symptoms observed after x-radiation and those noted in experimental adrenal cortical insufficiency; and 4, the reported reduction in mortality of x-radiated mice by the use of desoxycorticosterone acetate (5). In view of the non-specificity of the adrenal alarm response to stress, it seems reasonable to postulate a similar early increase in adrenal cortical activity after irradiation, which, if prolonged, may possibly result terminally in a relative adrenal insufficiency.

Many of the changes observed after x-radiation are apparently secondary to a direct effect of the radiation upon cells. Thus, Barnes and Furth (1), using parabiotic mice, have demonstrated a remote effect of radiation on nonirradiated tissues, including lymphoid structures and bone marrow. Similar observations have been made clinically following local irradiation of lymphoid tissue. That the adrenals are implicated in some of these indirect responses is suggested by the findings of Leblond and Segal (7). These investigators have described a generalized involution of lymphoid structures following heavy local x-radiation (2000 r–3600 r) in normal but not in adrenalectomized rats. After adrenalectomy, lymphoid changes were restricted to the irradiated area. Recently, Dougherty and White (4) have reported a lymphopenia and structural alterations in lymphoid tissue following 10 r in normal mice. Similar radiation doses given to adrenalectomized animals failed to evoke a lymphopenic response. Since larger doses (200 r) produced similar changes in intact and adrenalectomized animals, it was suggested that x-rays exert both direct and indirect effects on lymphocytes, the latter being mediated by a pituitary-adrenal cortical mechanism.

It was the purpose of the present investigation to determine the adrenal weight and the adrenal cholesterol content at frequent intervals following total body x-radiation at three selected doses, sublethal, LD_{50} and LD_{100}. It was hoped in this way to follow any alteration in adrenal activity during the course of radiation sickness and to provide thereby a possible basis for interpreting some of the physiological changes observed after x-radiation.

[1] This document is based on work performed under Contract no. W-31-109-eng-38 for the Manhattan Project at the Argonne National Laboratory.

METHODS. Two hundred and fifty white male rats (Maguran) ranging in weight from 210 to 285 grams received total body x-radiation in a single exposure at one of three dose levels, 200 roentgens (r), 650 r or 900 r. The radiation factors for the 200 r and 900 r exposures were: 200 kv, 15 Ma, 0.5 mm. Cu 1.0 mm. Al filters, 72.5 cm. distance, dose rate 15 r/minute. Radiation factors for the 650 r exposure were identical with the exception of the distance which was 101.3 cm. and the dose rate, 8 r/minute. In the 650 r experiment which was carried out first, the animals were exposed in cellulose acetate boxes in groups of 10. In the subsequent experiments animals were irradiated in large perforated aluminum boxes in groups of 8. It was believed that the aluminum box would not only be more convenient but would also provide better ventilation. Calculations indicated that absorption of x-rays by the perforated aluminum box is negligible.

In order to control the factor of selection, each group was designated after x-ray for a specific sacrifice time. Rats which died during the course of the experimental period were analyzed in the same manner as those which were sacrificed. All animals were weighed before and after irradiation and at the time of sacrifice or death.

Animals were sacrificed with nembutal (IP); and the adrenals, spleen and kidneys were removed, dissected free of fat and weighed on a torsion balance. The paired adrenals from single animals were macerated and digested according to the method described by Levin (8). Total adrenal cholesterol was determined by the Schoenheimer-Sperry method as modified by Sperry (14). The color developed in the Liebermann-Burchard reaction was read in the Evelyn photoelectric colorimeter using the 660 filter. In one series of experiments at 900 r, the adrenals, kidneys and a skeletal muscle were weighed before and after drying to constant weight in an oven at 120°C.

Two types of control groups were used. One group consisted of 68 untreated male rats, the other of 57 male rats which were placed in the exposure boxes for a duration equivalent to the length of time required for x-ray. The exposure box controls were sacrificed at different intervals after boxing.

RESULTS. The percentage changes in adrenal cholesterol and adrenal weight after total body x-radiation at 200 r, 650 r and 900 r are indicated in figure 1. A summary of all data is presented in table 1, a, b and c.

Adrenal cholesterol. Adrenal cholesterol expressed as milligrams total cholesterol per 100 grams of body weight is decreased by some 40 to 50 per cent 3 to 6 hours after x-radiation at either 650 r or 900 r. After 200 r, however, there is only a slight reduction in cholesterol (10–15 per cent) comparable to the change observed in the exposure box controls at the same time. Cholesterol rises somewhat in the 650 r group 2 days after x-ray and continues to increase reaching a level some 100 per cent above the normal at 7 days. This is followed by a return toward the normal at 9 and 11 days and a further increase at 15 days. It should be pointed out that many of the rats were dying in the 7 to 11 day period after 650 r. After the initial decrease in adrenal cholesterol with 900 r, there is a slight recovery followed by a marked fall at 3 days, amounting to about 75

per cent. A considerable number of the animals were prostrate and showed severe diarrhea, nasal hemorrhages, marked edema and hemorrhage in the gastro-intestinal tract at this time. The majority of deaths occurred around 4 days after irradiation with 900 r. Animals dying or sacrificed while moribund after either 650 r or 900 r show a greatly reduced adrenal cholesterol (80 per cent). In the 200 r group, there is a significant elevation in cholesterol content of the adrenals 2 days after x-radiation followed by a gradual return to normal. None of the animals died after 200 r.

Adrenal weight. Adrenal weight expressed as milligrams per 100 grams of body weight increases 6 hours after x-ray in both the 650 r and 900 r groups but

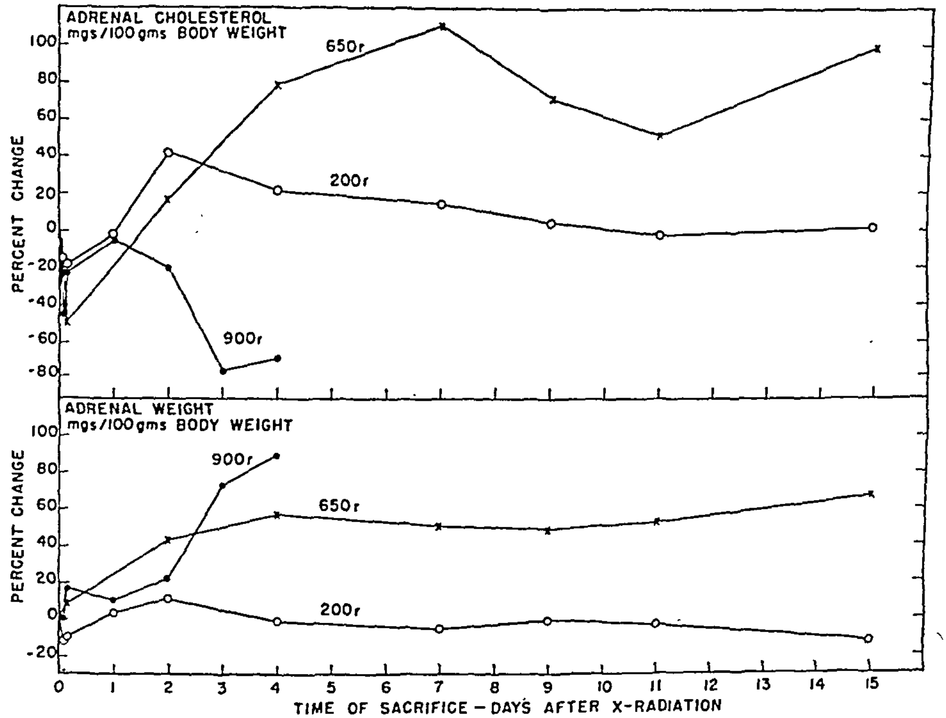

Fig. 1. Adrenal weight and cholesterol content after total body x-radiation.

a rather similar rise is noted in the exposure box controls at this time. At 2 days, however, adrenal weight is increased by some 20 to 40 per cent in the x-ray groups whereas no further rise is observed in the controls. In the 650 r group, the adrenals continue to show an increase in weight at 4 days (55 per cent increase). This is maintained at 7, 9, 11 and 15 days after x-radiation. A marked increase in adrenal size of some 75 per cent is observed 3 days after 900 r. The adrenals are even somewhat heavier at 4 days (90 per cent increase). An increase in adrenal weight of over 100 per cent is seen in animals from the 650 r and 900 r groups which died or were sacrificed while moribund. After x-radiation at 200 r little change is noted in adrenal size.

The increased adrenal wet weight observed after 650 r and 900 r is not a result of an increase in percentage of water, for the adrenal dry weight increases in a

TABLE 1a

Changes following x-radiation at 200 r

GROUP	SACRIFICE TIME	NUMBER OF ANIMALS	BODY WEIGHT GMS. Before x-ray	BODY WEIGHT GMS. At sacrifice	ADRENAL WT., MGM./100 GMS. BODY WEIGHT*	ADRENAL CHOLESTEROL Mgm./100 mgm. adrenal	ADRENAL CHOLESTEROL Mgm./100 gms. body weight*	SPLEEN WT., GMS./100 GMS. BODY WEIGHT*	KIDNEY WT., GMS./100 GMS. BODY WEIGHT*
	days								
Untreated controls		23		247.4	13.35 ± 0.38†	2.51 ± 0.16	0.33 ± 0.02	0.593 ± 0.039	0.793 ± 0.010
X-radiated	0.12	8	279.6	274.4	11.76 ± 0.53	2.43 ± 0.24	0.29 ± 0.03	0.543 ± 0.051	0.710 ± 0.048
X-radiated	0.25	8	262.9	256.7	12.08 ± 0.60	2.29 ± 0.23	0.28 ± 0.03	0.584 ± 0.053	**0.714** ± 0.026
X-radiated	1	8	271.9	260.6	13.86 ± 0.62	2.38 ± 0.28	0.33 ± 0.04	**0.389** ± 0.032	0.764 ± 0.031
X-radiated	2	8	275.8	260.9	14.84 ± 0.64	3.21 ± 0.33	**0.48** ± 0.05	0.478 ± 0.038	0.800 ± 0.021
X-radiated	4	8	277.0	278.3	13.21 ± 0.55	3.08 ± 0.18	0.41 ± 0.03	**0.348** ± 0.018	0.743 ± 0.015
X-radiated	7	8	262.5	270.0	12.71 ± 0.61	3.03 ± 0.38	0.38 ± 0.05	0.492 ± 0.032	0.767 ± 0.022
X-radiated	9	6	281.7	291.5	13.36 ± 0.91	2.56 ± 0.14	0.34 ± 0.06	**0.370** ± 0.033	0.743 ± 0.019
X-radiated	11	8	254.1	274.7	12.97 ± 0.68	2.59 ± 0.24	0.33 ± 0.03	0.446 ± 0.062	0.742 ± 0.027
X-radiated	15	8	261.3	286.5	11.73 ± 0.44	2.95 ± 0.29	0.34 ± 0.03	**0.327** ± 0.019	0.763 ± 0.018

TABLE 1b

Changes following x-radiation at 650 r

Untreated controls		25		235.5	12.87 ± 0.49	2.95 ± 0.11	0.38 ± 0.02	0.609 ± 0.026
Exposure box controls	0.25	10	248.3	243.0	14.12 ± 0.35	2.46 ± 0.17	0.35 ± 0.02	0.638 ± 0.049
Exposure box controls	2	9	261.2	264.3	12.01 ± 0.66	3.04 ± 0.17	0.36 ± 0.04	0.591 ± 0.058
X-radiated	0.25	10	261.9	252.2	13.98 ± 0.72	**1.42** ± 0.29	**0.19** ± 0.03	0.485 ± 0.032
X-radiated	2	10	216.9	194.2	**18.52** ± 1.19	2.48 ± 0.16	0.45 ± 0.01	**0.316** ± 0.019
X-radiated	4	10	215.4	175.9	**20.31** ± 1.11	3.44 ± 0.35	**0.68** ± 0.06	**0.321** ± 0.033
X-radiated	7	8	220.0	194.8	**19.44** ± 1.88	**4.21** ± 0.22	**0.80** ± 0.06	**0.263** ± 0.037
X-radiated	9	7	214.3	188.2	**19.29** ± 2.90	3.70 ± 0.46	**0.66** ± 0.07	**0.364** ± 0.042
X-radiated	11	8	220.0	187.1	**19.71** ± 1.74	3.07 ± 0.54	**0.58** ± 0.10	**0.295** ± 0.027
X-radiated	15	4	210.0	177.1	**21.60** ± 3.49	3.35 ± 0.57	**0.76** ± 0.24	0.474 ± 0.020
X-radiated	at death‡	11	213.5	167.9	**30.25** ± 2.52	**0.54** ± 0.12	**0.15** ± 0.03	**0.241** ± 0.019

TABLE 1c

Changes following x-radiation at 900 r

Untreated controls		20		257.9	13.14 ± 0.48	2.60 ± 0.20	0.34 ± 0.03	0.628 ± 0.047	0.780 ± 0.013
Exposure box controls	0.12	7	278.3	293.0	12.69 ± 0.73	2.15 ± 0.30	0.28 ± 0.05	0.541 ± 0.041	0.768 ± 0.015
Exposure box controls	1	8	273.1	265.6	14.02 ± 0.82	2.49 ± 0.23	0.35 ± 0.04	0.586 ± 0.045	0.795 ± 0.020
Exposure box controls	2	8	263.1	249.3	14.45 ± 0.08	2.43 ± 0.17	0.36 ± 0.04	0.651 ± 0.047	0.798 ± 0.043
Exposure box controls	3	7	281.4	287.2	12.53 ± 0.76	2.44 ± 0.12	0.31 ± 0.07	0.552 ± 0.022	0.784 ± 0.025
Exposure box controls	4	8	256.9	259.4	14.43 ± 1.14	2.37 ± 0.16	0.35 ± 0.05	0.543 ± 0.062	0.817 ± 0.049
X-radiated	0.12	8	272.6	264.5	13.23 ± 0.41	**1.44** ± 0.10	**0.19** ± 0.02	0.508 ± 0.022	0.777 ± 0.018
X-radiated	0.25	8	266.9	255.0	15.38 ± 0.53	1.74 ± 0.19	0.27 ± 0.03	0.552 ± 0.035	0.783 ± 0.027
X-radiated	1	8	270.5	248.4	14.53 ± 0.52	2.25 ± 0.27	0.33 ± 0.04	0.417 ± 0.055	0.740 ± 0.035
X-radiated	2	8	267.5	251.5	**16.14** ± 0.55	1.71 ± 0.26	0.27 ± 0.04	**0.322** ± 0.020	0.758 ± 0.028
X-radiated	3	8	258.8	222.3	**22.68** ± 1.26	**0.37** ± 0.06	**0.09** ± 0.02	**0.132** ± 0.007	0.721 ± 0.019
X-radiated	4	15	267.1	223.2	**24.89** ± 1.20	**0.47** ± 0.08	**0.11** ± 0.02	**0.146** ± 0.010	0.755 ± 0.023
X-radiated	at death	20	264.1	211.6	**27.65** ± 0.86	**0.26** ± 0.03	**0.07** ± 0.02	**0.152** ± 0.011	0.796 ± 0.020

* Body weight at sacrifice used in calculations.
† Indicates standard error of the mean.
‡ Includes 4 animals at death, 4 found dead and 3 sacrificed in moribund condition.
Boldface figures indicate statistically significant difference ($p < 0.01$) from mean of untreated controls.

rather similar fashion (fig. 2). The percentage water is thus almost equivalent at all sacrifice periods even though the wet weight is increasing. Kidney and muscle wet and dry weights were determined as controls. These were remarkably constant in water content after x-radiation (table 2).

Spleen, kidney and body weights. Although there is some indication of a decrease in spleen size[2] as early as 6 hours after x-radiation, these changes are

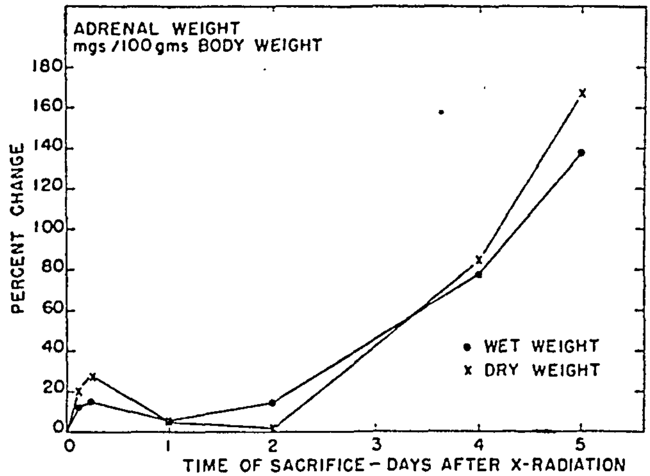

Fig. 2. Adrenal wet and dry weight after total body x-radiation.

TABLE 2

Per cent water in adrenal, kidney and muscle after x-radiation at 900 r

GROUP	SACRIFICE TIME	NUMBER OF ANIMALS	BODY WEIGHT AT SACRIFICE GMS.	ADRENAL WEIGHT, MGM./100 GMS. BODY WEIGHT			KIDNEY WEIGHT, GMS./100 GMS. BODY WEIGHT			MUSCLE PER CENT WATER
				Wet	Dry	Per cent water	Wet	Dry	Per cent water	
	days									
Untreated controls..		10	279.2	11.92	3.04	73.7	0.724	0.164	77.3	76.4
X-radiated.........	0.12	6	272.0	13.41	3.66	72.7	0.739	0.173	76.6	75.5
X-radiated..........	0.25	4	282.0	13.69	3.89	71.5	0.714	0.170	76.3	75.7
X-radiated.........	1	5	274.0	12.54	3.17	74.8	0.751	0.175	77.0	76.9
X-radiated.........	2	5	273.4	13.70	3.07	77.7	0.699	0.159	77.3	76.5
X-radiated.........	4	10	250.3	21.15	5.62	72.4	0.791	0.180	76.9	75.8
X-radiated.........	5	4	229.3	28.31	8.12	71.1	0.819	0.191	76.7	76.3

not significant until the 2 day sacrifice period at which time a 50 to 60 per cent decrease is noted in both the 650 r and 900 r groups. The spleen continues to decrease in weight in the latter group until death. In the 650 r group, there is also a further decrease in spleen weight with the maximum change observed at 7 days. This is followed by a gradual recovery at 9, 11, and 15 days. At death spleen has decreased in the 650 r and 900 r groups to roughly one-fourth of the

[2] The weight of the spleen in the controls is somewhat higher than usually described for other rat strains. This may be the result of a Bartonella infection which was discovered in a number of the stock rats after these studies were in progress.

control weight. A reduction in spleen weight is noted as early as 1 day after 200 r. This decrease is maintained at most of the subsequent sacrifice periods. It is interesting to note, however, that statistically significant weight changes ($p < 0.01$) are observed only at the 1, 4, 9 and 15 day sacrifice periods after 200 r.

Kidney weight expressed as grams per 100 grams of body weight shows no significant deviation in any of the groups after x-radiation with one exception. A small but significant decrease (10 per cent) is observed in the group sacrificed 6 hours after 200 r.

There is a small progressive decrease in body weight at each sacrifice period for the 650 r and 900 r groups. With 200 r there is a slight decrease (5 per cent) in body weight until the fourth day. Body weight then increases progressively reaching a level some 10 per cent above the pre x-ray weight at the 15 day sacrifice period. Exposure box controls and x-radiated animals show a similar small decrease in weight during boxing (2–4 per cent).

Discussion. The early decrease in adrenal cholesterol after total body x-radiation at 650 r and 900 r is similar in magnitude and time course to that following a single injection of adrenotrophic hormone in the intact rat (12). Comparable changes are also observed in many conditions of stress and are believed to be associated with an increased adrenal activity (8, 9, 12, 13). Since similar effects are produced by the injection of the adrenal cortical hormones and the pituitary adrenotrophic hormone when the adrenals are intact, it has been concluded that the adrenotrophic hormone actually increases the rate of secretion of the adrenal cortical hormones (12). Furthermore, since these effects of the adrenotrophic hormone are associated with a simultaneous reduction in adrenal cholesterol content, there is considerable likelihood that cholesterol is related to the formation of the adrenal cortical hormones which are also of a steroid nature.

The apparent increase in adrenal activity, indicated by a decrease in cholesterol content as early as 3 hours after irradiation, may account, in part, for some of the early symptoms of radiation sickness. Reference has already been made to the similarity in the blood picture and lymphoid tissue following x-ray and following the injection of adrenal cortical extract or pituitary adrenotrophic hormone. Likewise, the prevention by adrenalectomy of some of the physiological changes induced by x-ray would substantiate this hypothesis (7). The significance of the greatly increased adrenal cholesterol observed one week after x-radiation in the 650 r group is not clear. This may be a result of prolonged stimulation of the adrenal cortex, indicating either a maintained increased cortical activity or actually a decrease in activity due perhaps to a type of exhaustion. In this connection it is interesting to note that injection of pituitary adrenotrophic hormone over a period of 3 days also results in an elevated adrenal cholesterol (12). The return of adrenal cholesterol toward normal observed at 9 and 11 days after exposure to 650 r may be attributed to the fact that a considerable number of animals were dying in the 7 to 11 day period. The presence of one or two animals which would have died shortly with a low adrenal cholesterol could account for the decrease observed at this time (fig. 1).

After 900 r, the demand for cortical hormone is probably excessive. This is

suggested by the abrupt decrease in adrenal cholesterol associated with a greatly increased adrenal weight 3 days after irradiation. These findings are rather similar to those observed terminally and probably represent the extreme adrenal response to stress. Adrenal cholesterol determinations in animals found dead are slightly lower than in animals at death or in animals sacrificed in a moribund condition. Yet, even if one eliminates the former, the decrease is still marked and statistically significant ($p < 0.01$).

The changes in adrenal cholesterol and adrenal weight after 200 r would seem to indicate little adrenal response to this dose. Still, the small early decrease in cholesterol content and the rather considerable increase at 2 days may suggest some change in adrenal activity even at 200 r. The exposure box controls, however, show a rather similar early decrease in adrenal cholesterol, indicating that the caging per se may be a mild stress situation. It may be noted that a much greater adrenal response, approximately 80 per cent decrease in adrenal cholesterol at 8 hours after exposure, has been reported by Dougherty and White in mice after 200 r (4).

Involution of lymphoid tissue is associated with the adrenal response to stress. The decrease in spleen weight observed after exposure to x-ray may, therefore, be related to this adrenal response as well as to the well-known direct effect of radiation on lymphoid tissue. It is interesting to note, however, that rather similar early changes in spleen weight were seen at each dose level, although little adrenal response was obtained with 200 r.

Since it was desired to express the adrenal and spleen weights in terms of body weight, the kidneys were weighed as a possible control on the general effect of body weight changes on organ weights. It was thought desirable to include an organ which was believed to be unaffected, at least with regard to its weight, by x-ray. Kidney weights expressed in terms of body weight with only one exception are not significantly different in any of the groups at the various sacrifice periods, although body weight decreases by 10 to 20 per cent after exposure to 650 r and 900 r. Since there is a small decrease in body weight after x-radiation, the possibility remains that partial inanition might account for some of the described adrenal changes. This is rendered unlikely, however, for it has been reported that complete starvation for 3 days does not change the cholesterol content of the adrenals (8). Although complete inanition will produce adrenal hypertrophy, the evidence relating to partial inanition is equivocal (15). If rats are chronically underfed there is actually a decrease in adrenal weight which is relatively greater than the loss in body weight (15). The relatively brief duration of the 900 r experiment would seem to preclude any serious effect on adrenal size as a result of the small body weight loss described.

SUMMARY

The changes in adrenal cholesterol content and adrenal weight observed after total body x-radiation at 650 r and 900 r suggest a considerable adrenal response to these dose levels. After 200 r, there is little change in adrenal activity as judged by these indices of response.

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AN EXPERIMENTAL STUDY OF INTRAMUSCULAR PRESSURE MEASUREMENTS

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For many years the importance of muscle tone in facilitiating the return circulation has been emphasized. Henderson referred to this as the venopressor mechanism (1). Recently, failure of this mechanism has been said to explain the depletion of the effective circulating blood volume in clinical shock (2, 3, 4). Differences of opinion exist as to the accuracy of intramuscular pressure changes as ordinarily measured. The observations of Henderson (1, 2), Gunther (3, 4) and Hellebrandt (5) conflict with those of Hathway and Moore (6), de Vries and Milwidsky (7), and Wells *et al.* (8). In this study an attempt was made to evaluate the reliability and significance of intramuscular pressure determination.

Methods and procedure. Henderson's method (9) embodying the modifications suggested by Kerr and Scott (10) was first compared with the modification devised by Gunther (11). A needle from each apparatus was inserted into the posterior thigh muscles of a rabbit anesthetized with Nembutal. Similar comparisons were made using the human biceps. The results are given in table 1.

With the Gunther modification consistent results were not obtained in consecutive readings. More consistent results were obtained with the Kerr-Scott modification of the Henderson apparatus and this was the method used during the remainder of the study.

Briefly, the intramuscular pressure technic of Henderson records the force in millimeters that must be exerted by a water manometer to produce perceptible movement of the saline meniscus in a 2 mm. bore adapter attached to a no. 20 needle, with the end plugged and with three side holes, thrust into a muscle belly. In all the animal experiments here reported the skin overlying the muscle was split to permit direct visualization of the muscle being tested. A three-way stopcock facilitated filling of the saline-filled adapter for repeated determinations. The rabbits used were anesthetized with Nembutal.

It was found to be most important rigidly to control the position of the muscle and the needle inserted therein. Very slight alterations in the position of the needle within the muscle sharply altered intramuscular pressure readings. To prevent such movement appropriate means were taken to fix both the limb and the needle-muscle arrangement in all these experiments. Duplicate readings were always taken and the results recorded are the average of the readings.

The effects of the following procedures on the intramuscular pressure were studied in the rabbit: *a*, sciatic stimulation and section; *b*, occlusion of the

[1] The author is indebted to Drs. C. C. Guthrie and T. K. Kruse for much helpful advise and criticism.

abdominal aorta and/or the vena cava; *c*, spinal cord section; *d*, hemorrhage; *e*, curarization, and *f*, coramine administration. In man simultaneous observations of blood pressure were made before, during, and after surgical procedures performed under spinal anesthesia.

RESULTS. *Sciatic stimulation and section.* After freeing the nerve, applying electrodes, and fixing the position of the limb and the muscle-needle arrangement, the nerve was stimulated with a tetanic current of graded strength for 30 seconds. Intramuscular pressure readings were taken during the period of stimulation. Typical results from one of eight such experiments together with the subsequent effect of nerve section are given in table 2A. Hellebrandt, Crigler and Kelso (5) have indicated by diagram that in frog gastrocnemius and in human biceps, that with an increasing strength of stimulus there is a progressive fall in intramuscular pressure. Such correlation was not obtained in these experiments. It must be pointed out that the motion necessarily acompanying sciatic stimulation materially affects the intramuscular pressure readings obtained during this procedure.

TABLE 1

Comparison of the Henderson and Gunther IMP methods

READING	RABBIT		HUMAN	
	Gunther	Henderson	Gunther	Henderson
	mm. H_2O	*mm. H_2O*	*mm. H_2O*	*mm. H_2O*
1	68	112	40	80
2	42	120	80	84
3	50	116	32	84
4	110	108	68	78
5	140	116	102	84
6	140	108	140	90
7	70	114	140	80
8	60	112	128	86
9	110	108	140	82
10	54	120	62	80

Aorta-caval occlusion. By means of heavy silk ligatures placed about the abdominal aorta and vena cava and tightened and released through glass tubes inserted into the abdomen it was possible temporarily to occlude either of these vessels with the abdomen closed and without disturbing the muscle-needle relationship. Typical results from one of four such experiments are given in table 2B. No demonstrable effect on the intramuscular pressure was produced by occlusion of either or both of these vessels or after release of the occlusion.

Spinal cord section. After preliminary surgical preparation so that the cord could be cut without disturbing the intramuscular pressure apparatus, the cord was severed between the first and second lumbar vertebrae. Three experiments did not demonstrate any significant change in intramuscular pressure. Data from one of these experiments are given in table 2C.

Hemorrhage. Kiely, Hamilton and Gellhorn (12) noted that hemorrhage in

unanesthetized, decerebrate dogs leads to a sharp rise in intramuscular pressure which returns to normal on reinfusion of blood. Kleinberg, Swingle and Hays

TABLE 2

Intramuscular pressures under various procedures, in the rabbit

A

Sciatic stimulation and after sciatic section

OBSERVATION	TIME	IMP	OBSERVATION	TIME	IMP
	min.	*mm. H_2O*		*min.*	*mm. H_2O*
Control	1	114	Stim. coil 9	13	110
Control	2	110	Stim. coil 10	15	110
Control	3	118			
Stim. coil 5	5	180	Cut sciatic		
Stim. coil 6	7	112		17	120
Stim. coil 7	8	140		19	110
Stim. coil 8	11	90		20	102
				23	132
				25	122

B

Before, during, and after aorta-caval occulsion

OBSERVATION	TIME	IMP	OBSERVATION	TIME	IMP
	min.	*mm. H_2O*		*min.*	*mm. H_2O*
Control	1	84		40	60
Control	5	90		45	76
Occlude vena cava	6		Occlude vena cava and		
	10	82	aorta	50	
	15	96		55	86
	20	78		60	78
	26	80		80	82
Release vena cava	27		Release vena cava and		
	30	84	aorta	85	
Occlude aorta	31			90	88
	35	90		120	90

C

Before and after section of the spinal cord

OBSERVATION	TIME	IMP	OBSERVATION	TIME	IMP
	min.	*mm. H_2O*		*min.*	*mm. H_2O*
Control	1	134		10	110
Control	2	140		25	122
Cord cut	5			45	128
	7	128		60	112

(13) also noted that hemorrhage caused a sharp rise in intramuscular pressure which was maintained for several hours. On the other hand, following hemor-

rhage in dogs Hathway and Moore (6) observed no change in intramuscular pressure.

In this study six rabbits were slowly bled to death by cutting a mesenteric vein, without significant change in intramuscular pressure. In one of these animals intramuscular pressures were followed for six hours and no significant change occurred. Data from this experiment are given in table 2D.

TABLE 2–*Continued*

D

Before, during, and after hemorrhage and after death

OBSERVATION	TIME	IMP	OBSERVATION	TIME	IMP
	min.	*mm. H_2O*		*min.*	*mm. H_2O*
Control	1	88		40	90
Control	2	94		50	80
Steadily progressive hemorrhage	10	84	Death	60	76
	20	80		120	80
	30	82		240	78
				300	72
				360	86

E

Before and after coramine and curare and after death

OBSERVATION	TIME	IMP	OBSERVATION	TIME	IMP
	min.	*mm. H_2O*		*min.*	*mm. H_2O*
Control	1	102	Curare 0.2 cc. IV	30	110
			Curare 0.3 cc. IV	34	96
Coramine 1 cc. IV	2	110	Curare 2.0 cc. IV	37	
Coramine 1 cc. IV	6	100			
Coramine 1 cc. IV	12	106	Death	40	116
Coramine 1 cc. IV	14	100		65	106
				120	106
Curare 0.3 cc. IV	16	92			
Curare 0.3 cc. IV	22	106			

Coramine and curare. Coramine has been reported to increase the intramuscular pressure (Gunther, 3). This agent was administered to five rabbits without noting any significant effect on the intramuscular pressure. Curare was also found to be without effect upon the intramuscular pressure. The data from a typical experiment are given in table 2E.

Human experiments. In preliminary experiments with humans it was found that with voluntary contraction of muscles, and particularly those muscles having denser sheaths (the extensors of the wrist and knee) it was possible to raise the intramuscular pressure but such increases in pressure did not regularly correlate with the strength of contraction. As reported by Wells *et al.* (8) increasing the

venous pressure by an occluding cuff or tourniquet caused an increase in the intramuscular pressure in the biceps or gastrocnemius.

Because of Gunther's claim (4) that the intramuscular pressure was useful in detecting incipient shock, 14 patients[2] undergoing major surgical procedures had continuous observation of the arterial blood pressure, the venous pressure, and the intramuscular pressure before, during, and after the surgical procedure. No change was noted in the intramuscular pressure in the biceps or gastrocnemius after the induction of spinal anesthesia and there was no correlation during the surgical procedures between the intramuscular pressure and either the venous pressure or the arterial blood pressure. Coramine (nikethamide) which Gunther has found useful in elevating the intramuscular pressure was administered intravenously in 5 cc. doses to several of these patients. It was found to cause a sharp but short-lived increase in respiratory rate and volume, but there was no consistent effect upon the intramuscular pressure.

In three adults with post-cerebral thrombosis hemiplegia of several weeks' duration no significant difference was obtained in the intramuscular pressure in the gastrocnemius of the affected and the unaffected legs.

SUMMARY

Intramuscular pressure determinations by the Kerr-Scott modification of the Henderson technic showed relatively little change in the posterior thigh muscles of the rabbit as a result of nerve stimulation, nerve section, arterial and venous occlusion, hemorrhage, spinal cord section, and after death.

In human muscles with dense sheaths such as the extensors of the wrist and of the knee, voluntary contraction or local venous obstruction increased intramuscular pressure.

After spinal anesthesia no change was found in the intramuscular pressure in human gastrocnemius or biceps. In 14 surgical patients no correlation between the intramuscular pressure and either the venous pressure or the arterial blood pressure was observed.

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[2] Through the courtesy of Dr. J. W. Shirer and Dr. S. J. Glass.

THE EFFECTS OF ANOXEMIC ANOXIA ON EXCITABILITY, CONDUCTION AND REFRACTORINESS OF MAMMALIAN CARDIAC MUSCLE[1]

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A recent study of terminal electrocardiograms of experimental animals dying of anoxemic anoxia and in hemorrhagic shock (2) showed that cessation of normal sequential activity of the auricles and ventricles is brought about via one of two routes. In about one half of the anoxic animals the first departure from normal was failure of the pacemaker, often followed for a short period by A-V nodal rhythm and then total standstill. In the other half of the anoxic animals A-V conduction failure with dissociation or ventricular standstill occurred. Animals dying in hemorrhagic shock almost invariably showed the pacemaker failure pattern of termination which characterized one-half of the anoxic deaths.

The following experiments were designed to study the fundamental functional properties of excitability, conduction, and refractoriness of mammalian heart muscle under varying degrees of oxygen lack from mild anoxia to the minimal level consistent with continued life, i.e., breathing about 5 to 7 volumes per cent O_2 in inspired air. It was believed that such information would provide more basic interpretations for the phenomena observed in heart failure.

PREPARATION. Dogs were used in all experiments. They were anesthetized with morphine and barbital sodium. The chest was opened by a midline incision of the sternum. The heart was suspended in the opening by suturing the cut edges of the ventral incision of the pericardium to the body walls, forming a cradle.

Artificial respiration was administered via a tracheal cannula. While the chest was being opened, and while the animal was receiving a normal atmosphere, artificial respiration was produced by the common technique of intermittently opening and closing a valve in the compressed air line by a small vacuum-driven motor (windshield wiper).

Rebreathing apparatus. An entirely new and simple arrangement of apparatus, not hitherto described, was devised for the administration of artificial respiration while rebreathing from a respirometer, and thereby reducing the oxygen content of the respired air. A diagram of the apparatus is shown in figure 1. It is a basal metabolism machine modified to make possible its use with artificial respiration. To the original apparatus was added the tank T which encloses the movable bell B. The tank T is airtight except for a small leak where the thread passes through its top, a regulated leak valve L, and the tube R which connects to an intermittent pressure artificial respiration apparatus similar to the one used

[1] This investigation was aided by a grant from the Ella Sachs Plotz Foundation.

during the preliminary operations when rebreathing is not desired. In some experiments the same intermittent pressure respirator was used for the preliminary and control periods and the rebreathing period also. The respiratory tube which was connected to the tracheal cannula during the period prior to rebreathing was shifted to *R* for rebreathing. The tracheal cannula was then connected at *A*. Intermittent positive pressure within the tank *T* periodically inflated the lungs. Deflation occurred as pressure in *T* dissipated due to the regulated leak *L* or the side tube on the respiratory tube attached at *R*. The tracheal cannula and the tubes communicating with the interior of the bell *B* must be leak proof.

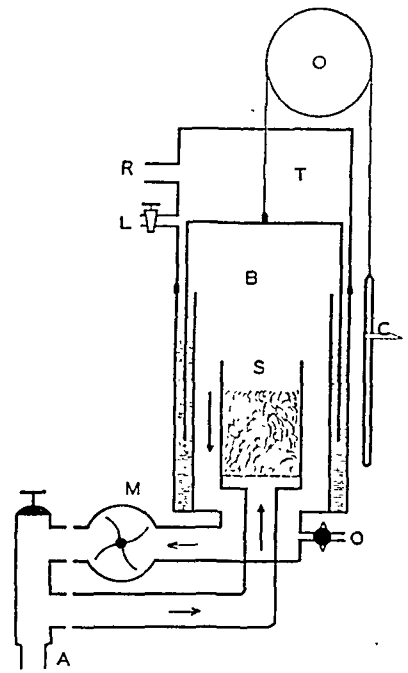


Fig. 1. Diagram of simple rebreathing apparatus consisting of a Sanborn basal metabolism apparatus with a motor circulator and the added tank *T* enclosing the oxygen bell. The intermittent pressure artificial respiration machine which attaches at *R* is not shown. See text for further description.

To facilitate deflation it was found advantageous to add fifty grams to the counterweight *C*, somewhat overbalancing the weight of the bell.

At the beginning of each experiment the bell *B* was filled to its maximal height with fresh air. The cannula was connected at *A* before pressure was admitted at *R*. By periodic measurements of the oxygen concentration it was possible to construct an approximate scale of percentages of O_2 for ready estimation by comparison with the position of the stylus on the kymograph at *C*. In every experiment estimates from such a scale were verified from time to time by analysis of a gas sample. Oxygen could be added at *O* at a regulated rate to maintain any desired oxygen concentration. Carbon dioxide was absorbed by the soda lime *S*.

For use with dogs or cats the basal metabolism machine must be equipped with a motor driven circulator. With the flapper valve system neither the natural

respiration of the animal (with closed chest) nor the artificial respiration provides sufficient circulation to remove carbon dioxide and maintain a uniform mixture of oxygen.

Stimulating and recording methods. An electronic discharge stimulator was used in experiments on excitability and on the duration of refractoriness. The shock was in the form of a spike, almost entirely monophasic (opposite phase about 5 per cent of main spike) and about 3 msec. in duration.

The arrangements for delivering testing stimuli to the ventricle at the desired point in the cardiac cycle and for recording responses from local contiguous bipolar leads (3) are diagrammatically illustrated in figure 2. The cam wheels *A* and *B* operated the keys to the inductorium which drove the heart at a controlled rate by stimuli applied to the right auricle at *D*. The S-A node had previously

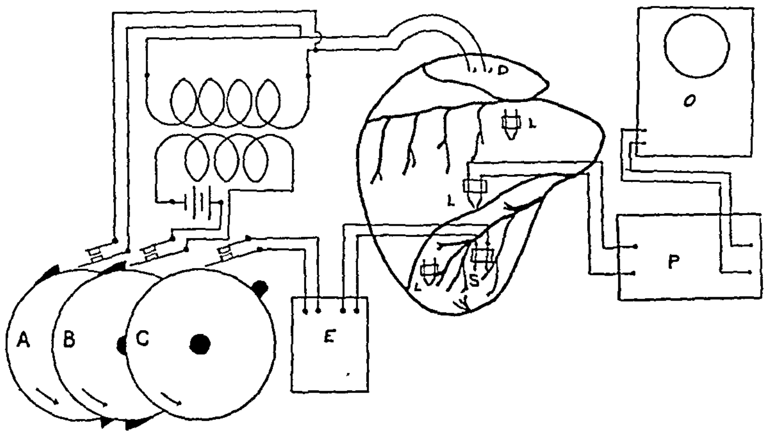


Fig. 2. Apparatus for driving the heart at a controlled rate, stimulating the ventricle at the desired moment in the cardiac cycle, and for recording from local leads on the ventricular surface. *A* and *B*, cam wheels to control the delivery of break shocks to the right auricle through driving electrodes *D*; *C*, cam wheel to trigger stimulator discharge to ventricular electrodes, *S*; *L*, local leads to pre-amplifier, *P*; *O*, Du Mont 208-B Cathode Ray Oscillograph. G.E. Electrocardiograph not shown.

been clamped. *B* operated the make-break key, and *A* short-circuited the make shocks. The cam on *C* operated a key to trigger the discharge of a stimulus to the ventricle. The timing of this testing stimulus was fully adjustable with respect to the driving stimulus from the break at *B*. This exact control of the timing of testing stimuli was necessary in experiments on the duration of refractoriness and of shock-spike intervals for the estimation of conduction changes in anoxia. In the testing of excitability it was found that comparable results were obtained when shocks were timed to fall late in the T-P interval and when they were allowed to fall at random from a self-excited oscillator at a rate two to three times the cardiac rate. The stimulating electrodes *S* were at various times placed upon different portions of the ventricular surface, but in the majority of experiments they were on the anterior surface of the left ventricle.

Leads for recording of local electrograms are indicated by the electrodes labelled *L*. In some experiments three or four local electrode assemblies were used

simultaneously. Only one could be recorded at a time but they could quickly be changed by turning a selector switch. The potentials were led to the Grass preamplifier P and then to the amplifier of the Du Mont 208-B cathode ray oscillograph. The self-excited sweep apparatus of the oscillograph was not used. The wiring was changed to provide for horizontal deflection of the action potentials, and the movement of the photographic paper provided the time axis. An L-II electrocardiogram was recorded by a G.E. electrocardiograph simultaneously with the local electrogram in all experiments.

RESULTS. *Excitability.* The thresholds of ventricular muscle to electrical stimulation in hearts supplied with normally oxygenated blood, during progres-

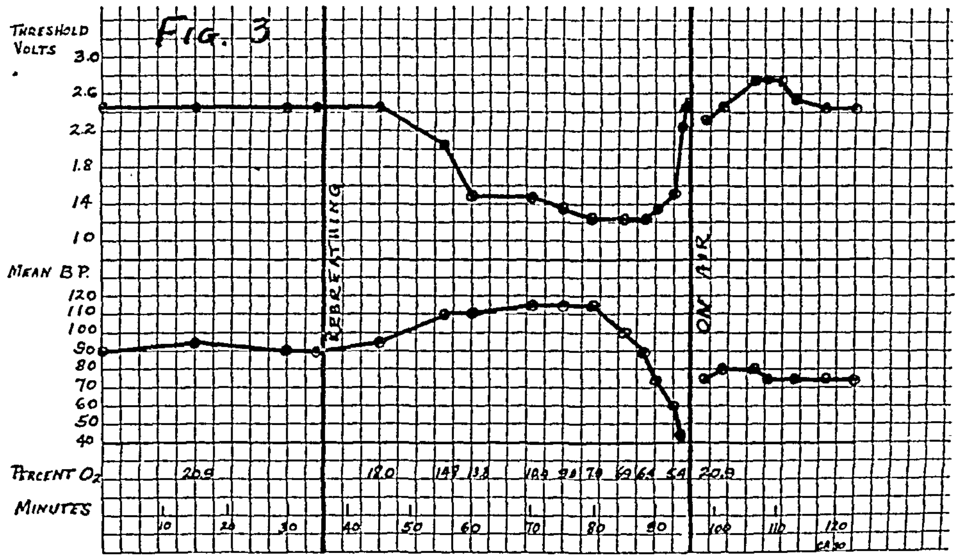


Fig. 3. Graph illustrating the changes in threshold of ventricular muscle and the changes in mean arterial pressure accompanying the various degrees of reduction of oxygen in inspired air.

sive stages of anoxemia and during recovery from the effects of severe anoxemia, have been studied in careful detail with brief (3 msec.) electronic discharges.

The pattern of excitability changes observed is graphically illustrated and correlated with blood pressure and oxygen concentrations in figure 3. The threshold changes were *a*, reduction in threshold voltage during moderate anoxia, the reduction persisting until a very severe anoxic state was reached when *b*, the threshold rose rapidly. The voltage indicated as the threshold at any point is the voltage which produced ventricular responses to about one-half of the test shocks. Each point during the control period prior to rebreathing represents the result of repeated readings. The variation between the strength of stimulus required to give an occasional response and that which produced a response upon each stimulus was about ten to fifteen per cent of the threshold value as defined. In the first points on the figure the threshold voltage (one response out of every two stimuli) was 2.45 volts. Rare responses occurred at 2.25 volts and responses were regular at 2.57.

After rebreathing was started and the oxygen concentration of inspired air thereby gradually reduced, significant threshold changes became evident at about 15 per cent O_2. As the reduction in O_2 concentration progressed the threshold fell rapidly and then more gradually. The minimal reading of 1.26 volts, representing almost a 50 per cent reduction, was found at oxygen concentrations between 9.0 and 6.4 per cent. Below 6.4 per cent the threshold rose very rapidly, and from the results of other experiments it is known that the voltage threshold would quickly have risen to a value three or four times the original if air had not been given at this point to save the animal for the study of thresholds during and after recovery from anoxia.

During the first ten minutes of recovery the threshold rose, reaching a plateau at 2.78 volts. This was maintained for about six minutes, after which the threshold gradually returned to the original reading of 2.45 volts, reaching it about twenty minutes after air respiration was restored.

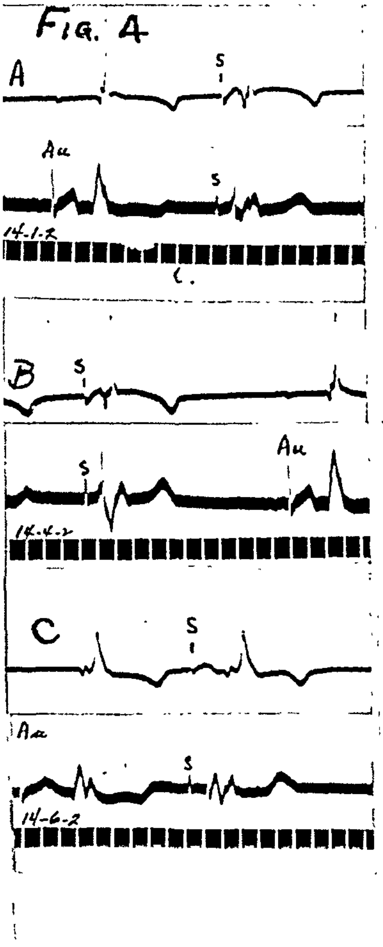
A comparison of the excitability curve with the mean blood pressure curve is of interest. With developing anoxia the blood pressure increased during the period of rapid decrease of threshold. Since this heart was driven at a constant rate of 120 per minute, the increase in pressure is due to factors other than heart rate (see discussion).

The rise of cardiac threshold in severe anoxia does not coincide with the decline of mean blood pressure, but follows it. The curves show that the beginning of the decline of blood pressure preceded the first rise of threshold by about eight minutes, and that the steep rise of threshold began when the blood pressure had reached about 50 mm. Hg on its rapid descent. It appears probable that the decline in blood pressure contributes to the cause of the elevation of threshold and may precipitate its onset. However, the progressively anoxic state of the muscle undoubtedly would have led to loss of excitability somewhat later even if the arterial pressure had somehow been maintained at a high level.

Conduction. Directional and semi-quantitative estimates of changes in conduction rate in anoxia have been made from three kinds of data: *1*, shock-spike intervals from local leads; *2*, P-R intervals, and *3*, duration of QRS.

These changes as derived in an illustrative experiment are shown in figure 4 and the measurements are given in table 1. The stimuli to the ventricle were delivered on the anterior surface near the apex and the local lead illustrated is from a placement on the right ventricle 41 mm. distant. In the control record *A*, made during respiration of air with normal oxygen content, the shock-spike interval was 65 msec. In record *B*, made during moderate reduction of oxygen to 11.4 per cent in inspired air, the shock-spike interval was shortened to 60 msec. In record *C*, made in severe anoxia produced by reduction of oxygen in inspired air to 6.5 per cent, the shock-spike interval was lengthened to 110 msec.

Changes in P-R intervals (from auricular shock Au to the beginning of *R*) and in duration of QRS are similar in direction to the changes described for ventricular shock-spike intervals, i.e., the durations were shortened during moderate anoxia and lengthened during the severe stage. In a very short time, possibly one minute after record *C* was made, a 2:1 heart block became evident.



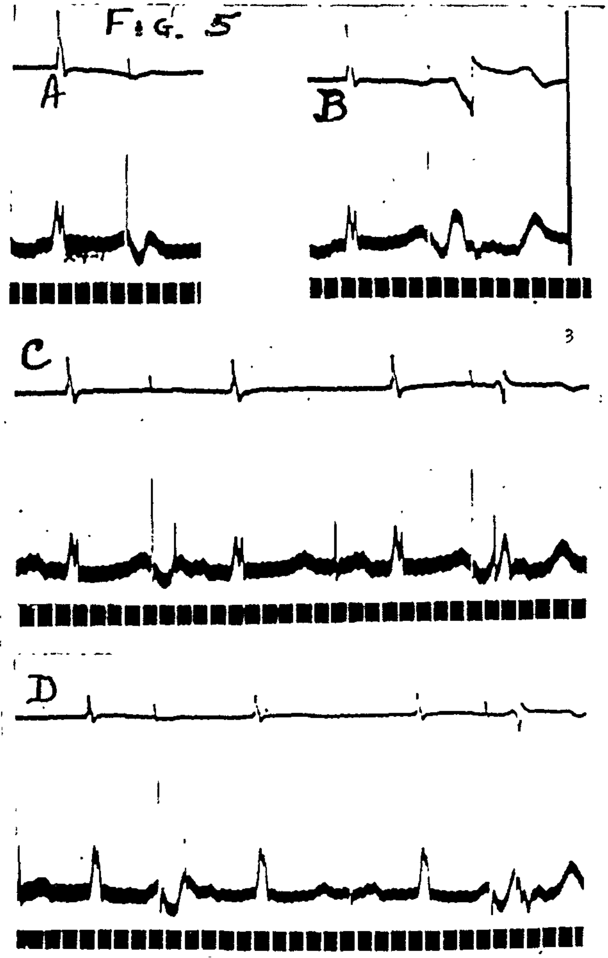


Fig. 4. Changes in shock spike intervals, P-R intervals, and duration of QRS in *A*, the control; *B*, moderate anoxia; and *C*, severe anoxia. *Au*, auricular or driving stimulus; *S*, ventricular stimulus.

Fig. 5. Measurement of the duration of refractoriness. *A* and *B*, control trials; *C*, moderate anoxia; *D*, severe anoxia. The small vertical lines on the local electrograms (upper line in each record) and the tall sharp spikes in the *T* wave of the ECG's are the artefacts of the testing stimuli. Time intervals 0.20 and 0.04 sec.

TABLE 1

Changes in shock-spike intervals, P-R intervals, and in duration of QRS during moderate and severe anoxia (intervals in milliseconds)

MINUTES OF REBREATHING	PERCENT O_2	MEAN B.P.	SHOCK-SPIKE INTERVAL	P-R	DURATION OF QRS
0	20.9	90	65	98	37
23	11.4	100	60	92	34
38	6.5	40	110	130	54

All of these changes in intervals may be regarded as indices to conduction changes in the heart, although the complexity of cardiac conduction makes im-

possible any statement of an absolute conduction rate. The changes in duration of intervals agree in indicating that in moderate anoxia there is a small increase in the rate of conduction, while in the severe stage the rate markedly decreases before total failure of cardiac function supervenes.

The shock-spike interval is made up of two components, latency of response at the stimulated locus and conduction. It is possible that changes in both contribute to the shortening of the interval in moderate anoxia and perhaps to the lengthening in severe anoxia. Since the maximal shortening of shock-spike interval is less than 10 per cent, it is probable that the greatest acceleration of conduction is somewhat less. The amount of shortening of the P-R interval and of duration of QRS are less than 10 per cent also.

The lengthening of all intervals in the severe stage is far greater in magnitude than the shortening in the moderate stage. Maximal increases of 75 to 100 per cent occur before 2:1 A-V block or dissociation occur.

The notch on the falling limb of the R wave of the ECG (fig. 4) is worthy of mention as it also changes with the conduction changes described. Such notches are seen quite often in electrocardiograms made with the chest open and the ventral surface of the heart exposed to the air. The notching logically is assumed to be due to the loss from the ECG of potentials produced by those portions of the ventricular muscle which are no longer in contact with tissues. This includes a large fraction of the surface muscle which receives the excitatory impulse at a time which is intermediate between the early and late portions (3). It includes much of the early responding muscle also, but a notch often results, nevertheless. In moderate anoxia during the period of accelerated conduction the notch becomes narrower and less obvious, but in severe anoxia with slowed conduction the potential components become separated so far that they appear as two distinct R spikes.

Duration of refractoriness. In reporting measurements of refractoriness it is of value to describe the stimuli used since the apparent duration of the absolute refractory period will vary with the duration and voltage of the testing stimuli. A monophasic stimulating potential applied to heart muscle stores a charge in the tissues (polarizes the tissues) about the electrode contacts. This charge decays along an exponential curve (5). With strong D.C. stimuli of 12 to 15 msec. duration the charge is of sufficient magnitude and decays with sufficient slowness to evoke a response at any time during an appreciable period (maximum of more than 100 msec. possibly) before the decay curve asymptotically approaches zero. The length of the period during which an excitation could result is a function of the voltage of the applied monophasic stimulus and undoubtedly of its duration also. A strong D.C. stimulus which is applied during the latter part of systole while the tissue is incapable of response can therefore cause the tissue to respond again as soon as refractoriness has passed. Records showing the results of such stimuli applied during systole have demonstrated that responses can occur after a delay or long latency. Records using the stimulating electrodes as leads to the recording galvanometer have shown that this was a true delay of response, and not to be attributed to slow conduction (5). In measuring the

duration of refractoriness, therefore, it is necessary to guard against errors that might result from the use of strong stimuli (usually monophasic or predominantly so) to test for the end of the absolutely refractory period.

In these experiments to be reported, errors due to polarization charges have been minimized, or standardized, by choosing arbitrarily a strength of stimulus which is three times the threshold voltage during late diastole in the normally oxygenated heart and with a duration of 3 msec. With these stimuli a stored charge of stimulating value will be very brief in duration and therefore the refractory period determined will closely approximate the true absolutely refractory period, e..cept for the limitation below.

In measuring the duration of refractoriness, the interval between the onset of R of the ECG and the moment of delivery of the earliest stimulus which would evoke a premature systole was sought. Since conduction of the natural impulse is involved, this does not measure the duration of absolute refractoriness in the tissues under the stimulating electrodes, but since the placement of electrodes is constant throughout an experiment, the *changes* in refractoriness are accurately shown in records made in this manner.

Figure 5 illustrates the method used in these experiments and the results in one. The upper electrogram in each section is a cathode ray oscillograph tracing recorded from local leads 1 cm. from the stimulating electrodes. The lower electrogram in each section is an L-II ECG. At first the apparatus was adjusted to deliver shocks in mid-diastole or middle of the T-P interval. Then in the succeeding minutes, records were repeated with the stimuli a few msec. closer to the summit of T in each trial than in the preceding one until responses no longer occurred.

The small vertical lines in or near the local lead T waves and the sharp tall spikes in the T wave of the ECG are simultaneous artefacts produced by the testing shock.

The R-shock interval in 5A is 166 msec. No responses were obtained at this interval in control trials. The intervals were lengthened again by gradual adjustment of the apparatus until responses to about one half of the shocks were obtained. 5B shows a response in the control series. The R-shock interval is 199 msec. This is taken as the duration of the refractory period.

Measurements at two stages of oxygen deficiency are illustrated by 5C and 5D. In each case the exploratory approach was carried out as in the control. In 5C, made at the 10 per cent oxygen level, the refractory period (responses to half of shocks) was found to average 202 msec. There is no significant difference between this and the 199 average of the control. Figure 5D, made at the 7.2 per cent O_2 level when the heart was dilated and the blood pressure falling, the refractory period was found to have shortened to 164 msec. Our measurements indicate that spontaneous variations are not greater than ± 5 msec. In severe anoxia this experiment shows a shortening of the refractory period by about 35 msec. Two other experiments yielded similar results. One other showed no shortening, but records at the most severe stage could not be measured because of distortions in the ECG. Two experiments showed a small amount of shortening

(12 to 16 msec.) in moderate anoxia and a greater amount (about 30 msec.) during the severe stages. No explanation for the inconsistent results in the moderately anoxic stages can be given.

DISCUSSION. All of the observations upon excitability, conduction, and duration of refractoriness in moderate anoxia are in agreement in indicating that the functional capacity of the heart muscle is not impaired. On the contrary, the enhancement of excitability and slight increase in rate of conduction may be interpreted as compensatory improvements. This view is strengthened by the finding previously well known that the blood pressure usually is elevated above the control even when the heart rate is held constant. Other evidences of compensatory reactions of the heart and circulatory system in moderate anoxia have been reviewed and discussed by Wiggers (8), citing especially the observations of Sands and DeGraff (6), and of Strughold (7). In addition to the increase in cardiac rate when not experimentally controlled, there is increased velocity of ejection. A reflex increase in vasomotor tone (1) contributes to the rise in arterial pressure.

The enhancement of excitability of cardiac muscle may be due in part to the effect of oxygen lack upon the muscle itself and in part to sympathetic excitation. Our experiments do not differentiate between the intrinsic and extrinsic possibilities. Lehmann (4) has shown that excised mammalian nerves subjected to total anoxia exhibit lowered thresholds during the first six to eight minutes, after which the threshold rises and excitability disappears. It is improbable that the process of excitability differs in fundamental nature in the different excitable tissues. Therefore, if one assumes the liberty of applying observations on nerve to excitability changes in cardiac muscle we can conclude that all or part of the lowering of threshold in anoxic dog hearts is an intrinsic change.

The increase in rate of conduction and the abbreviation of systole are changes that are known to result from sympathetic stimulation and injected adrenalin (9). Since there is strong indirect evidence that sympathetic excitation of the heart occurs in anoxia (6), the acceleration of conduction and the shortening of refractoriness observed in this study could have been due to sympathetic impulses and secreted epinephrine. Lehmann's paper reported that excised nerves conduct more rapidly in moderate anoxia. Therefore, the sympathetic factors and anoxic changes within the muscle both may have contributed to the observed phenomena.

Severe anoxia (below 8 per cent) is very dangerous. The danger resides largely in the fact that in anoxia there is no recognized simple sign that cardiac failure is imminent though it may be only a few minutes away. The arterial blood pressure which is so often used as an indicator of the condition of animals and patients can be of little help unless the readings are continuous. Circulatory collapse may come with great rapidity in anoxia. Even with the chest open, the heart under direct observation and the blood pressure manometer always in the range of vision, we often were unable to anticipate the onset of rapid functional deterioration with sufficient accuracy to allow ourselves the three or four minutes required to record a full set of records from four or five local leads. At the end of our attempts to complete a set of records during this crisis stage, we found upon a

number of occasions that revival was no longer possible even after changing the respiration to normal air and applying cardiac massage. The difficult revivals were in trials in which the change back to air was delayed until the mean blood pressure had fallen to a level below 30 mm. Hg. In these cases the hearts remained markedly dilated for some time and contracted feebly. In some of these attempted revivals the hearts grew feebler and stopped. In others they gradually gained force and appeared normal after 20 to 30 minutes though the blood pressure seldom regained the control reading. If the switch to air was made while the mean blood pressure was as high as 40 mm., recovery was prompt though the pressure sometimes was established at a new level somewhat lower than the original control.

From such observations one gains the impression that the heart uses its anerobic sources of energy to practically complete exhaustion by the time the rapid fall in blood pressure reaches the 30 mm. level. Recovery is hindered or prevented by the lack of perfusion of the heart adequate to restore its chemical energy sources.

Previous studies have shown that hearts which fail due to overall anoxia cease their effective pumping by reason of either pacemaker stoppage or cessation of A-V conduction (2). By the time that these changes become manifest, weakness of contraction is evident also. The heart is soft to the touch and empties itself to only a small degree. No objective recording of muscular tension was made in our experiments, but in the optical arterial pressure curves of Sands and DeGraff (6) the rapid decline in mean pressure is accompanied by a great reduction in pulse pressure. With a constant heart rate and reduced aortic size (assuming unchanged elasticity), this may be regarded as evidence of a reduced systolic discharge. Therefore it appears that diminution of contractility accompanies the slowing of conduction, rise of threshold and pacemaker stoppage which characterize cardiac failure in severe anoxia.

Neither ectopic ventricular systoles nor ventricular fibrillation occurred in any of the thirty dogs used in this series of experiments. Although the excitability of the heart muscle to electric stimuli was increased considerably during moderate anoxia, the increase, evidently, was not sufficient to produce ectopic discharges. The rise of threshold during the terminal period would seem to reduce the probability of ectopic systoles.

The abbreviation of the refractory period would, if of sufficient extent, make possible re-entrant conduction and the establishment of fibrillation. However, the refractory period was not reduced to a duration of less than about 160 msec. while the heart retained sufficient excitability for tests to be made. It has been found in studies upon the initiation of fibrillation that a reduction to about 80 msec. together with a considerable slowing of the conduction rate is required for the establishment of ventricular fibrillation in the heart of the dog (5).

SUMMARY

Methods for the production of a slowly developed and accurately controlled anoxia and for testing the excitability, conduction changes, and the duration of refractoriness are described.

During moderate anoxia the excitability of the dogs' ventricles to brief shocks delivered at a constant moment in diastole was found to be increased (threshold lowered). In some experiments the maximal change in threshold was near fifty per cent. In severe anoxia after the blood pressure curve entered its steep decline the threshold rose rapidly reaching levels two or three times the control within a brief period.

Changes in conduction rate were indicated by changes in the duration of various intervals which include conduction time. Stimulus-response intervals (from local leads), P-R intervals and the QRS of the electrocardiogram all were shortened somewhat during moderate anoxia. All were lengthened rapidly and markedly when the "crisis" was approached in severe anoxia.

The duration of refractoriness was shortened in severe anoxia by about 30 msec. or fifteen per cent. In some experiments a smaller degree of shortening was recorded at moderately anoxic levels.

No ventricular ectopic systoles were observed or recorded in this series of experiments. This together with the smallness of the reduction in the refractory period account for the absence of ventricular fibrillations in anoxemic anoxia.

Because of the suddenness of the collapse in severe anoxia in many experiments and the lack of specific signs warning of imminent cardiac failure, it is regarded as very dangerous.

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THE USE OF ACETYLCHOLINE IN THE OBJECTIVE DETERMINATION OF CIRCULATION TIME AND THE FRACTIONATION OF THE VASCULAR BED TRAVERSED[1]

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While definite limitations to the utility of the circulation time exist, this procedure has nevertheless served a useful purpose as a simple laboratory adjunct in evaluating the state of the circulation (1). One of the deficiencies inherent in most methods of circulation time determinations is the subjective character of the end point. This has been recognized and a large variety of agents yielding objective end points have been recommended. Some, like radium-C (2), diodrast (3), fluorescein (4, 5), methylene blue (6), radioactive sodium (7) and thorium-X (8), have the handicap of requiring special equipment for their use. Others, like histamine (9), sodium cyanide (10, 11), lobeline, (12, 13), papaverine (14) and aminophylline (15) are found to be not entirely satisfactory either because of the unusually long circulation time values obtained, or because the end point is not distinct, or because the test substance tends to be toxic. For these reasons objective methods have not been widely employed and reliance has continued to be placed on the subjective methods.

Our recent studies in the dog with acetylcholine[4] indicate that it provides an excellent agent for the objective measurement of circulation time, the end point being denoted by the temporary depression of the sinus node or A-V junction. The end point employed was the slowing of the heart rate as revealed electrocardiographically in the anesthetized dog or in the blood pressure record in the unanesthetized dog. In this study an attempt was made to obtain the value of the circulation time in the unanesthetized dog and, in the anesthetized dog, to localize the site of the objective end point.

The Circulation Time in the Unanesthetized Dog. Methods. Twenty-two normal unanesthetized dogs weighing 9 to 16 kgm. were employed. These were fasted for 20 or more hours prior to the determinations. With the animals lying in the right recumbent position, acetylcholine chloride in doses of 1 to 25 mgm. (0.02 to 0.5 cc. of a 5 per cent solution) was rapidly injected into the foreleg vein via a 20 gauge needle connected to a tuberculin syringe. Because of the small volume the injection occupied only a fraction of a second. Care was taken to permit only a minimum quantity of blood to enter the syringe since blood cholinesterase rapidly destroys acetylcholine. The blood pressure was

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recorded continuously from the femoral artery by the Hamilton method (16), before, during and after the injection, the time of injection being indicated by an interruption of the base line (fig. 1). In the early determinations the larger

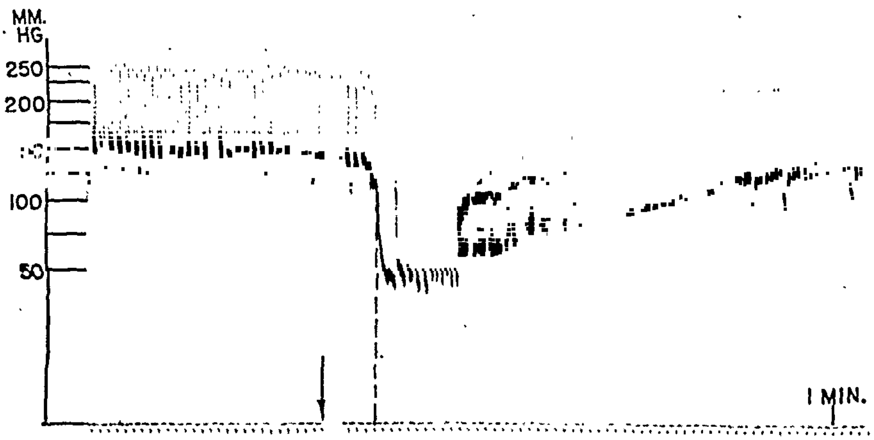

Fig. 1. Blood pressure recorded with Hamilton manometer in unanesthetized dog showing the effect of injection of 2.5 mgm. of acetylcholine into the foreleg vein. Ordinates show the calibration in millimeters of mercury. Abscissae indicate time in seconds. Arrow indicates time of onset of injection. The circulation time is 6 seconds (from arrow to perpendicular dropped to time line). The period of complete asystole is 2 seconds.

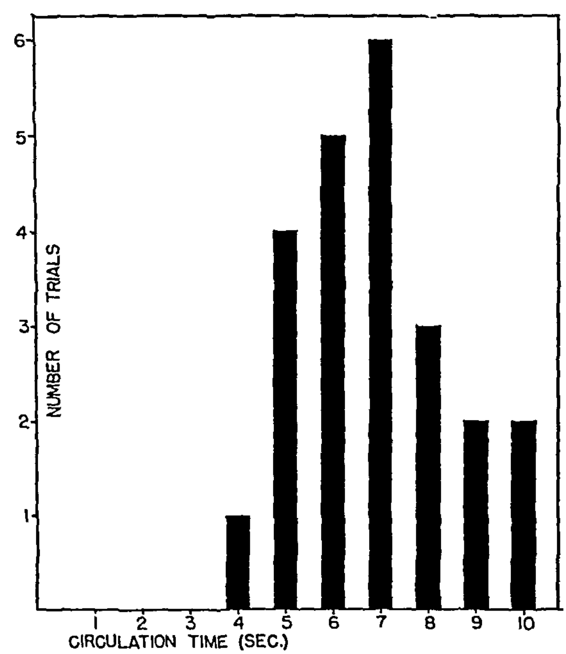

Fig. 2. Distribution of the circulation times obtained with acetylcholine in the intact unanesthetized dog following injection into the forelimb vein. All ½ second values were converted to the subsequent whole number for the sake of clarity.

doses were employed but in later tests this was reduced since it was found that the smaller doses gave equally effective results.

Results. Figure 2 summarizes the distribution of the circulation time. The mean obtained was 6.7 seconds and the distribution curve is fairly symmetrical.

The range of values found was 4 to 9½ seconds. The duration of the asystole lasted from 1 to 6 seconds, and the cardiac slowing somewhat longer. The maximum depression of blood pressure occurred in 10 to 14 seconds and the pressure drop lasted 41 to 340 seconds. No serious reactions were obtained. Fleeting side effects such as salivation, urination and slight to moderate excitement occurred in 5 animals. Frequently a respiratory gasp or grunt appeared simultaneously with or immediately following the onset of asystole. This was generally followed by a tachypnea of a few seconds' duration.

The Site of the End Point. Methods. Seven dogs weighing from 9 to 15 kgm. were anesthetized with pentobarbital sodium (25 mgm./kgm.) The chest was opened and artificial respiration instituted. Acetylcholine was injected as in the previous experiments, using 2.5 mgm. (0.05 cc. of a 5 per cent solution).

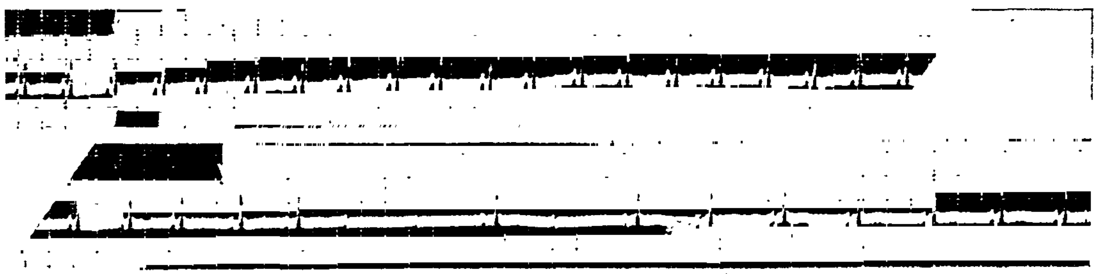

Fig. 3. Acetylcholine circulation time recorded electrocardiographically (lead 2) in the anesthetized open-chested dog. The first arrow denotes the time of injection in the superior vena cava of 2.5 mgm. of acetylcholine. The second arrow indicates the end point, at which the regular auricular response is expected but fails to appear. (Sometimes the end point is taken where an expected ventricular response fails to appear.) In this instance the circulation time is 8.5 seconds. A brief period of complete A-V block is noted immediately after the slowing of the auricles. This is followed by a partial A-V block. The slowing of the sinus node persists until the end of the record. The duration of ventricular asystole is 1.6 seconds.

A continuous electrocardiogram was taken (lead 2) to record the circulation time (fig. 3). The time of injection was indicated on the record by a simultaneous depression of the lead marker. The circulation time was measured from the onset of injection to the point when an expected auricular or ventricular response failed to occur on time. The injections were made into the superior vena cava, right ventricular cavity, main pulmonary artery, left ventricular cavity (near the apex), root of the aorta—in the region of the sinuses of Valsalva, the aorta—2 or 3 cm. above its origin, high in the ascending aorta, the transverse arch of the aorta, the descending thoracic aorta, the common carotid arteries, and the myocardium of the right and left ventricles. Bilateral vagotomy was later performed in four of the animals, following which injections were repeated in the superior vena cava, main pulmonary artery and root of the aorta. In 2 animals a loose cotton ligature was placed about the right coronary artery, 1 to 2 cm. from its origin, following which acetylcholine was rapidly injected into the main pulmonary artery, and the ligature drawn taut to occlude completely the coronary vessel. In all, over 160 injections were made in this series of experiments.

Results. The pertinent data are summarized in table 1. It was found that injections high in the ascending aorta, in the transverse arch, in the descending thoracic aorta and in the carotid arteries yielded no end points. Injections 2 or 3 cm. above the root of the aorta resulted in a more prolonged circulation time than injections made in the region of the sinuses of Valsalva. This suggested that the acetylcholine traveled via the coronary arteries to the sinus node and A-V junction. Generally, these structures are supplied through rami of the right coronary artery (17). Accordingly, in several experiments the right coronary artery was occluded immediately following the injection of acetylcholine into the main pulmonary artery. No end point occurred following this coronary occlusion initially, supporting the hypothesis. However, when this maneuver was repeated after a period of several minutes following occlusion of the right coronary artery, an end point again occurred, suggesting the opening of anastomatic channels between the right and left coronary arteries. Injection directly into the myocardium of the right and left ventricles produced no end point.

TABLE 1

Circulation time values †2 (sec.) obtained in the anesthetized open-chested dog by injection into various components of the vascular bed traversed

ANIMAL	SUP. VENA CAVA			RT. VENT.			MAIN PULM. ART.			LEFT VENT. (APEX)			ROOT OF AORTA			2-3 CM. ABOVE ROOT OF AORTA		
	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.
1	5.6	7.5	6.4				5.1	6.0	5.7				1.4	2.1	1.7			
2	7.4	12.5	9.9				5.7	8.8	7.5							3.0	6.3	4.3
3	6.4	8.4	7.1				3.0	5.4	3.9				1.2	1.6	1.4			
4	7.2	7.7	7.5				6.7	8.6	7.6							2.7	2.7	2.7
5	8.0	11.8	9.6				5.7	6.4	6.0							2.5	2.8	2.6
6	5.4	7.3	6.7			4.4	3.8	4.2	4.0	2.1	2.2	2.1	0.8	1.4	1.1	1.9	3.7	3.1
7	5.6	8.1	6.5	6.4	7.3	6.8	4.5	6.0	5.0	1.4	2.4	1.7	1.4	1.9	1.6			
Average*...			6.7			6.0			5.2			1.8			1.3			3.4

* Average of total trials performed.

† To first dropped beat (see text for discussion).

Sometimes the standstill affected only the ventricles. Usually, however, auricular standstill occurred. Cardiac standstill generally persisted from 1 to 7 seconds. Auricular fibrillation following acetylcholine was not infrequent and was paroxysmal in character. In the presence of auricular fibrillation the circulation time was found to be definitely greater than with sinus rhythm, indicating that auricular fibrillation per se prolonged circulation time. In 1 animal the circulation time from the superior vena cava averaged 9.6 seconds during the sinus rhythm, while during auricular fibrillation it averaged 11.2 seconds. Similar observations have been made in man (18).

Frequently in the anesthetized open-chest animal as in the unanesthetized one evidence of respiratory stimulation was manifest. This occurred within 2 to 5 seconds following the injection of acetylcholine. Section of the vagi abolished these heaving respiratory efforts.

It is to be noted from table 1 that approximately 50 per cent of the circulation time represents passage of acetylcholine through the large cross-sectional area of the pulmonary bed. It is evident that the time occupied by acetylcholine in this passage from a systemic vein to the specialized tissue in the heart would partition in a similar order.

Discussion. Acetylcholine appears to offer a clear end point for the objective determination of circulation time. There is need for such an objective method. Thus it has been shown (19) that smoking, old age and previous strong stimulation of the taste buds may lead to excessive prolongation of circulation time or failure to obtain an end point in those methods dependent upon taste or heat perception.

The small volume of acetylcholine offers certain advantages. The duration of injection and the volume of agent injected have been demonstrated to exert considerable influence on the length of the circulation time. Thus Loevenhart *et al.* (10) found that sodium cyanide in dogs and rabbits gave a prolonged circulation time when the injection period exceeded 1 second. Ruskin and Rockwell (19) obtained shortened thiamine circulation time when they increased the volume of the thiamine solution despite constancy of dosage. This false low value was attributed to the propulsion factor due to injection under pressure through a wide bore needle. The low figures obtained with diodrast may therefore be, in large part, artefact rather than an accurate measure of the circulation time. The small volume and the short period of injection (a fraction of a second) in the acetylcholine method avoids these errors.

TABLE 2

Comparison of values for circulation time obtained in the dog with acetylcholine, sodium cyanide and fluorescein

AGENT	VASCULAR CIRCUIT	CIRCULATION TIME (SEC.)		
		Min.	Max.	Av.
Acetylcholine	Foreleg to S-A or A-V nodes	4.0	9.5	6.7
Sodium cyanide				
Loevenhart et al. (10)	Ext. jugular vein to carotid sinus	5.4	13.8	8.7
Olsen et al. (20)	Femoral vein to carotid sinus	6	15	9–13
Fluorescein (5)	Femoral vein to conjunctiva	9	16	12.6

Another advantage of the acetylcholine method is its simplicity. The acetylcholine method can be performed electrocardiographically, or by cardiac auscultation or pulse palpation, using the first prolonged beat as the end point. Preliminary data in man suggest the possibility that the appearance of a cough may also be employed as an end point since it is almost simultaneous with, and a frequent accompaniment of, the cardiac slowing. The electrocardiographic technique is considered preferable since the measurement can be checked and the data filed for investigative purposes.

In table 2 a comparison is made of the circulation times obtained with acetylcholine and those reported in the dog with sodium cyanide (20) and fluorescein (5). It is evident that acetylcholine yields a distinctly shorter circulation time

and shows less variation. It would appear that acetylcholine is superior to most methods applicable to animals because of its simplicity, accuracy and the consistent and unequivocal nature of the end point.

It must be emphasized that inasmuch as acetylcholine is very rapidly destroyed by blood cholinesterase, only the minimum quantity of blood necessary to establish venepuncture be drawn into the syringe and that injection be made promptly upon entering the vein. This susceptibility to blood cholinesterase tends to eliminate the hazards of the drug since it is unlikely that an appreciable period of asystole could develop. Depression of the P-Q segment and a change in P wave contour after acetylcholine injection sometimes preceded the slowing of the heart used as an end point. However, these P-Q and P wave changes were inconstant compared to the change in rate and therefore not suitable for circulation time measurements.

SUMMARY

Circulation time studies with acetylcholine were performed in 22 unanesthetized normal dogs and 7 anesthetized open-chest animals. The end point was the transitory inhibition of the sinus node or, occasionally, the A-V junctional tissue. In the unanesthetized dog, records were obtained with the modified Hamilton manometer in order to observe simultaneous blood pressure responses. Circulation time from foreleg to sinus node or A-V junction was found to range from 4.0 to 9.5 seconds, averaging 6.7 seconds. In the anesthetized animal the vascular bed was fractionated by injection of the agent into its various components and recording the events electrocardiographically. The average circulation time (to the first dropped beat) from the superior vena cava was 6.7 seconds, from the right ventricle 6.0 seconds, from the main pulmonary artery 5.2, from the left ventricle 1.8, from the root of the aorta 1.3, and when injection was made 2 to 3 cm. above the sinuses of Valsalva the average interval was 3.4 seconds. Injection high in the ascending aorta, transverse aorta or in the carotid arteries yielded no cardiac end point. Injection into the main pulmonary artery followed by immediate ligation of the right coronary artery likewise resulted in no end point on the initial trials, indicating that the agent operated by a direct effect upon the sinus node.

The figures obtained by this method are smaller than those obtained with other procedures, for reasons presented in the discussion, and indicate that a more objective and accurate measure of the circulation time is secured with acetylcholine.

It is felt that, owing to its simplicity and greater objectivity, the acetylcholine method may be superior to other available procedures.

We are indebted to Dr. L. N. Katz for his valuable advice and criticisms in the conduct of this study.

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THE EFFECTS OF DIETARY CALORIC RESTRICTION ON MATURITY AND SENESCENCE, WITH PARTICULAR REFERENCE TO FERTILITY AND LONGEVITY[1]

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In this paper the results of experiments designed to test the effects of long-time caloric restriction upon the reproductive capacity and life-span of female A-strain mice are reported. Studies on the effects of pure caloric restriction over long periods of time are difficult to perform and the results require cautious interpretation. The observations here reported appear to indicate that female A-strain mice maintained for 240 days on diets containing amounts of protein, vitamins and minerals calculated to be identical with their litter mate controls, but restricted in carbohydrate and fat so that their calorie consumption was reduced to about two-thirds of the controls, were relatively infertile during that period. Upon institution of *ad libitum* feeding these animals promptly became fertile, and remained so for several months, at a time when the full-fed controls were practically sterile, presumably due to senescence. The average life span of female mice maintained throughout life on the calorie-restricted regimen was markedly greater than that of the animals fed *ad libitum.*

The fact that general and specific diet deficiencies cause suppression of reproductive functions is well known. Papanicolau and Stockard (1920) showed that underfeeding for short periods caused a reversible inhibition of estrus in rats. Evans and Bishop (1922) found that partial inanition or deficiency in vitamin B complex in rats causes a cessation of the estrous cycle. Coward and Morgan (1941) showed that deficiency in thiamine alone produced this effect in rats. However, Drill and Burrill (1944) showed that the anorexia associated with thiamine lack could account for the results on the basis of reduced total food intake. White and Andervont (1943) observed that in the mouse a low-cystine diet produced anestrus. White and White (1944) noted a similar response to lysine deficiency.

The effects on female mice of the C_3H strain of caloric deficiency, in the presence of protein, vitamin and mineral intake approximately equal to normal controls, were studied by Ball, Huseby and Visscher (1943) and Huseby, Ball and Visscher (1945). It was found that such restriction caused a decrease in the frequency of estrous cycles, an infantile uterus and vagina and a failure of mammary gland growth. These changes were found to be associated with the presence of ovaries containing large numbers of follicles in all stages of develop-

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ment. The general picture resembled that found after hypophysectomy and suggested that the changes in the ovary might be secondary to an anterior pituitary effect, and that the end organ alterations might therefore be in whole or in part a third order effect of calorie underfeeding. White *et al.* (1944) also found estrus-inhibition to result from caloric restriction.

The effects of partial inanition and caloric-underfeeding of rats upon longevity have been studied by McCay and his co-workers (1935, 1939a, b, 1943), Will and McCay (1943) and Riesen, Herbst, Walker and Elvehjem (1947). Studies on mice were reported by Visscher, Ball, Barnes and Sivertsen (1942). In general it has been found that animals on the lower calorie intakes outlived their litter mate controls. However, numerous factors enter into the results. They will be discussed later.

Experimental procedure. One hundred and forty-four A strain female mice were separated at weaning into two equal groups, one of which was placed on the calorie-restricted diet, and the other consisting of their litter mate sisters

TABLE 1
Composition of the diets

COMPONENT	CONTROL DIET, GRAM/100	RESTRICTED DIET, GRAM/100
Glucose	33.0	22.0
Casein	28.0	37.4
Lard	17.0	12.3
Yeast	8.0	10.6
Alfalfa leaf	4.0	5.4
Salt mixture*	7.0	9.3
Cod liver oil	2.0	2.0
Wheat germ oil	1.0	1.0

* $CaCO_3$—543, Mg CO_3—25, Mg SO_4—16, NaCl—69, KCl—112, KH_2PO_4—212, $FePO_4$—21, KI—0.08.

was fed the control diet *ad libitum.* The diet compositions for the two groups are shown in table 1. These two diets are so constructed that when the consumption of the restricted diet is held at three-fourths of that of the control, the calorie intake is reduced approximately one-third, while the intakes of protein, vitamins and minerals for the two are identical. The actual average food intake of the two groups of mice over the first 120 days of life is shown in figure 1. It will be seen that the experimental design was not perfect in that the food intake of the controls during the period 20 to 60 days was more than the anticipated four-thirds of the restricted. For the next sixty days the average observed values approached those planned. The intake of the restricted group was held at 1.51 grams per day throughout the remainder of the experiment. The food consumption of the controls was measured by weighing the food cups at each filling. The food offered the experimental group was measured daily for each mouse, using a series of hollowed-out brass molds, cut to hold the desired quantity by weight of the food mixture. Repeated calibration showed that ac-

curacy to $\pm$ 5 per cent can be obtained by this method. Food was compounded at two week intervals. The non-spill feed cups employed were 2 inch ointment tins with tops which had a circular hole just large enough to admit the head of an adult mouse. All mice were individually housed in small galvanized iron wire cages with solid tops and bottoms, cleaned and sterilized weekly. Tap water was continuously available. The animals were kept in an air-conditioned room at a temperature of 78$\pm$ 4°F and relative humidity of 45 $\pm$ 10 per cent saturation. Body weights were recorded daily. When the restricted females were approximately four months of age they were mated daily by placing males of known virility in their cages after the female had consumed her daily ration of food, and left together overnight. The male was removed before the next feeding. Thus no complication in food intake was introduced. When a restricted female became pregnant her litter mate control was mated. In this way the number of pregnancies in the two groups was kept the same. All litters were removed at birth.

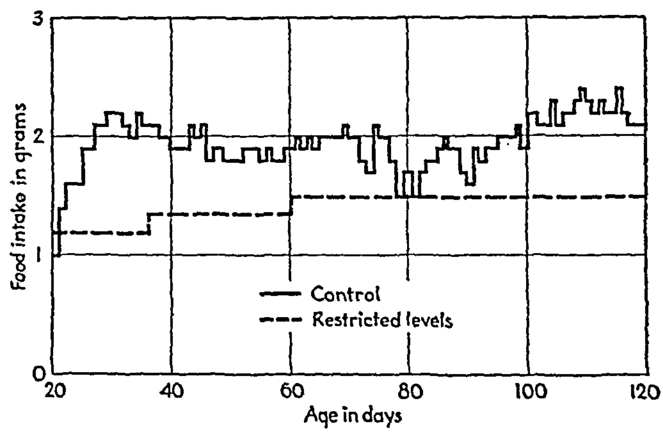

Fig. 1. Food intake of control and calorie-restricted A strain mice.

At 240 days twenty-six of the calorie-restricted mice selected at random from the total living at that time were fed the control diet *ad libitum*. They were again exposed to potent males and as they became pregnant their litter mates in the original control group were also mated. The decline in fertility in the full-fed animals at this point in the life span made it impossible to maintain equal numbers of pregnancies from this time forward.

At death each animal was dissected grossly and tumors were studied by microscopic section.

RESULTS. The weight records of the mice treated as indicated above are shown in figure 2. It is to be noted that the average body weights of calorie-restricted animals fluctuated around 17 grams during the period after 60 days, while the *ad libitum* controls showed a rising curve, broken at 260 days by an unfortunate epidemic of a generally non-fatal[4] respiratory infection, reaching 28 grams at 340 days. The weight response of the calorie-restricted animals to

[4] See the mortality data below to verify this assertion.

subsequent full feeding at 240 days was dramatic. Within 40 days these mice attained the same body weight as their litter mates who had received the control diet *ad libitum* since weaning.

Figure 3 shows a part of the results of the studies on fertility of A strain females in relation to caloric restriction. Several striking facts are evident. Although every normal control of the 20 tested was fertile up to 240 days, after that period only 20 per cent, or 4 of 20 mated, were capable of becoming pregnant on repeated tests. In the calorie-restricted group 31.3 per cent of the 64 mated daily became pregnant at least once in the early months, while only 12.5 per cent of 32 tested were fertile later. However, within two weeks of the beginning of *ad libitum* feeding after 240 days of caloric restriction every female of 26 so treated became pregnant.

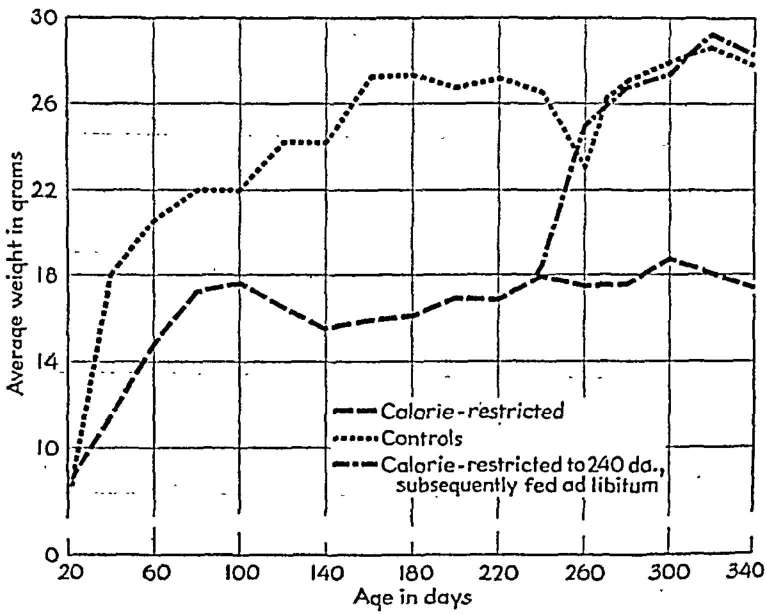

Fig. 2. Average body weights of full-fed control, calorie-restricted, and restricted-refed A strain mice.

The differences are much more striking when the total number of pregnancies obtainable is taken into account. If one divides the total number of pregnancies in a given group of mice by the number of mice and by the number of months, one obtains a fertility index, representing the average number of pregnancies per mouse per month. Taking the period from 243 to 374 days for the restricted, the restricted-subsequently full fed, and the fully fed control groups, the values are 0.04, 0.65 and 0.05 respectively. The index for the period 115 to 241 days in the restricted group is 0.08. The original data from which these figures are calculated are shown in table 2.

It can be said therefore that on the diets employed in the study A strain females restricted approximately one-third in calories for 240 days and subsequently fed *ad libitum*, had 13 times as many litters in the next 4 months as did their control litter mates fed *ad libitum* from weaning.

The data concerning life span are shown in figure 4. It is evident that under the conditions of this experiment calorie-restricted mice tended to outlive their litter-mate sisters. Caloric restriction to 240 days of age with subsequent full feeding was associated with a slight, but not definitely significant, increase in life span. It should be noted that all cancer deaths are eliminated from the data in figure 4, by removing such animals from each series. The cancer incidence in the several groups is shown in table 3. It will be noted that there was a zero incidence of cancer in the life-time calorie-restricted group. The relatively low cancer incidence in the fully fed breeders is believed to have been

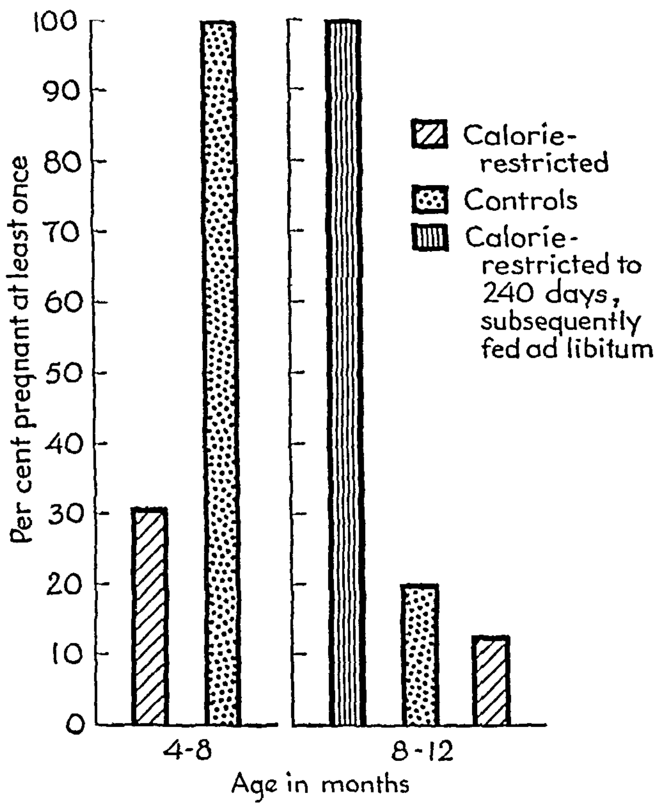

Fig. 3. Percentage of A strain mice becoming pregnant at least once at various ages in relation to dietary treatment.

the result of the fact that the mice bore on the average only slightly more than one litter apiece. It may be noted that the average cancer age is higher in the restricted-refed animals than in the control breeders.

The effects which have been described are believed to have been brought about by caloric restriction. Two points require notice in this regard. One is the fact mentioned earlier, that the restricted animals were fed, during part of their life spans, amounts of protein, vitamins and minerals somewhat less than their fully fed controls received. The amount fed the experimentals was fixed on the basis of observations of mean food intake of normal A strain females in a preliminary group of studies.

However, the restricted animals received amounts of protein, vitamins and minerals entirely adequate for normal growth and function in the presence of adequate calories. Therefore the results are unquestionably a result of caloric restriction. It is uncertain, however, whether the same degree of caloric restriction with a higher absolute protein intake might not yield a quantitatively different result. It is possible that with more protein it would require greater

TABLE 2

Breeding history in relation to caloric restriction

	AGE IN DAYS			PREGNANT AT LEAST ONCE
	115–190	191–241	243–374	
				%
Calorie-restricted				39.1
No. mated	64	57	32	
No. pregnant once	20	1	4	
No. pregnant twice	0	1	1	
No. pregnant 3 times	0	0	0	
Restricted and refed				100.0
No. mated after refeeding	—	—	26	
No. pregnant once	—	—	26	
No. pregnant twice	—	—	20	
No. pregnant 3 times	—	—	17	
No. pregnant 4 times	—	—	10	
No. pregnant 5 times	—	—	1	
Controls I*				100.0
No. mated	20	1	—	
No. pregnant once	20	—	—	
No. pregnant twice	—	1	—	
Controls II†				20.0
No. mated	—	—	20	
No. pregnant once	—	—	4	
No. pregnant twice	—	—	0	

* Bred only after each litter mate in calorie-restricted group had become pregnant.

† Bred only after each litter mate in restricted and refed group had become pregnant.

— Indicates that animals were not bred for a particular period.

0 Indicates no litters after total number had been mated repeatedly with males of known virility.

caloric restriction to produce the degree of infertility seen in the underfed mice in this study.

The second point to be noted is that whenever the non-protein calories are insufficient to meet the basal and activity energy requirements, protein itself will be burned for fuel. In this connection it should be mentioned that the calorie-restricted mice in these experiments were obviously more active than their controls. No quantitative activity measurements were made but the

restricted animals spent so much more time climbing up and down their cage walls and doors than did the controls that any observer could identify the group of animals on restriction simply by noting which were more active in this regard. Thus caloric restriction may aggravate the tendency to burn protein for fuel. When calorie intake is lowered to the point that this occurs it is no longer mean-

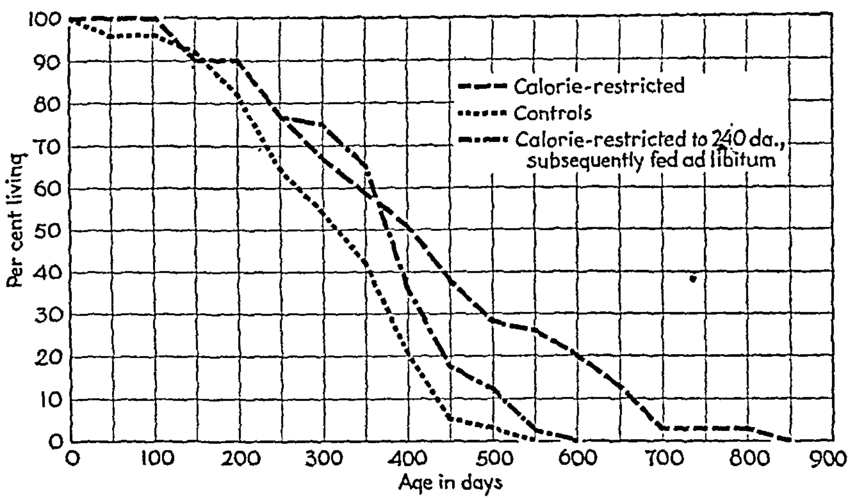

Fig. 4. Mortality data on A strain mice in relation to dietary treatment.

TABLE 3

Mammary cancer incidence on control and calorie-restricted diets

	NUMBER OF MICE	NUMBER OF TUMORS	PER CENT TUMORS	AVERAGE TUMOR AGE
				months
Control				
Breeders	21	4	19	11.1
Virgins	51	2	4	16.6
Restricted				
Breeders*	21	0	0	—
Virgins†	43	—	—	—
Restricted-refed				
Breeders	26	5	19	15.7
Virgins‡	0	—	—	—

* Mice becoming pregnant one or more times.

† Mice exposed repeatedly to males of known potency but did not become pregnant.

‡ All mice in the restricted-refed group became pregnant.

ingful to speak of *simple caloric restriction* because there is also an amino acid deficiency for processes of growth and repair. This problem poses a dilemma which cannot be resolved on the basis of the facts at hand. For the time being therefore the simple descriptive facts must be accepted and the interpretation in more fundamental terms must be left for future investigation.

The dietary caloric restriction employed in these studies produced a state of

near-sterility in the mice. The fertility index of 0.08 between the ages of 115 and 141 days is about ten per cent of the usual value found at the same age in fully-fed A strain females in this colony. No figure for the latter quantity is available in this study because of the design of the experiment. The prompt increase in fertility index to 0.65 upon full-feeding indicates that the caloric restriction served essentially to delay the occurrence of sexual maturity, without diminishing the capacity for maturation, to any large degree at least. There is no evidence in these observations that the 8 month old calorie-restricted mouse is much different in reproductive capacity from a pre-puberal full-fed animal. These observations give indication that caloric restriction simply retards sexual maturation rather than leading to an abnormal state.

However, the fact that the survival rate of the animals was definitely diminished by full-feeding after 8 months of restriction indicates that such treatment did not prevent early senescence from occurring. If the mortality data from eight months onward are considered it will be seen that 50 per cent of the surviving controls died in 125 days. Half of the surviving calorie-restricted mice died in 200 days, while half of the restricted re-fed animals died in 140 days. Furthermore the striking longevity of a few of the life-duration calorie restricted group did not appear in either of the other groups. It would appear that *ad libitum* feeding in this experiment was incompatible with long survival in aged mice. The question of whether early restriction appreciably lengthened the life span in females subsequently full-fed cannot be answered by the results of this experiment. The slight effect, noted above, may be due to random variation; on the other hand, it may be a real effect. Furthermore it must be recalled that this group bore on the average 3 litters apiece, while the controls bore an average of only 1 litter apiece. Since litter-bearing itself reduces the life expectancy, according to Murray (1936), the slight prolongation seen here may be an indication that virgins so treated might show a greater effect on longevity. No experimental test of this question has been made in this study.

After this study was underway it was evident that autopsies after death would not permit significant statements as to cause of death except from malignant tumors. All animals dying with mammary carcinoma are eliminated from statistical consideration because it is known from the studies of Visscher, Ball, Barnes and Sivertsen (1942), Tannenbaum (1942), and others, that caloric restriction greatly lowers breast cancer incidence. No other tumors were noted with sufficient frequency to permit an evaluation of the effects of the experimental procedures upon their incidence. All of the statements made above refer to deaths due to causes other than mammary carcinoma. The most important question, namely, what types of pathological process associated with senescence were delayed in appearance by calorie underfeeding, remains for further study.

SUMMARY

1. Calorie underfed female mice of the A strain show a fertility index of 0.08 litter per mouse per month during the period to 8 months of age, and 0.04 thereafter.

2. When previously underfed females are allowed food *ad libitum* at 8 months of age their fertility index rises to 0.65 litter per mouse per month, which approximates the normal for young full-fed females.

3. It is concluded that maturation with respect to reproductive capacity is retarded or postponed when low calorie feeding is imposed through a large fraction of the average normal life-span. The calorie underfeeding did not prevent subsequent maturation and normal gestation at an age when full-fed littermate sisters had become nearly sterile from senility, showing a fertility index of only 0.05 litter per mouse per month.

4. In the experiments reported, 25 per cent of A strain female mice subjected to life-time caloric restriction were alive at an age when all of their full-fed littermate sisters had died.

5. Considering the survival beyond 240 days in these experiments, the 50 per cent mortality times for the life-time calorie-restricted group, the 8 month restricted, subsequently full-fed group, and the full-fed controls were 200, 140 and 125 days respectively. Some factors influencing the significance of these observations have been discussed.

6. The caloric restriction employed in these studies retarded sexual maturation but did not prevent its occurrence upon subsequent full-feeding, and was associated with a tendency toward a longer life-span.

7. Caloric restriction for 240 days, with subsequent full-feeding, did not prevent a significant incidence of mammary carcinoma although it increased the average cancer age above that in comparable non-virgin, fully fed controls.

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THE EFFECT OF ADDING BORON TO A POTASSIUM-DEFICIENT DIET IN THE RAT

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Studies dealing with the substitution of one inorganic element for another in physiological processes have not been extensive. Most such experiments have dealt with either the interrelationships of the alkaline earth metals or substitutions of single elements in enzyme systems. An example of the former line of approach is the observation that rubidium and cesium may partially replace potassium in the diet of the growing rat (1, 2). Morphological studies have further indicated that myocardial and renal necroses, which are so characteristic of potassium deficiency in animals, may be modified when either rubidium or cesium is added to a potassium-deficient regimen. Of particular interest, therefore, is a report (4) which indicates that boron may have a beneficial effect on rats fed a diet of low potassium content; for this reason the following morphological observations have been carried out.

Methods. The basal potassium-deficient diet consisted of casein (Labco), 18; lard, 10; sucrose, 67.8; salts (5), 4 and choline, 0.2. Fifteen drops of viosterol were added to each kilogram of diet. Crystalline vitamins[2] were administered orally three times weekly, each animal receiving the following amounts per week: thiamine chloride, 0.2 mgm.; riboflavin, 0.28 mgm.; calcium pantothenate, 1.0 mgm.; pyridoxine, 0.2 mgm.; inositol, 0.1 gram; and para-aminobenzoic acid, 0.5 gram. Alpha tocopherol, 1.0 mgm. per animal, was given weekly.

Albino rats weighing 40 to 50 grams were used and were divided into the following groups: *Potassium-deficient*, basal diet alone, 9 rats; *potassium-deficient boron added*, basal diet plus 1.0 per cent borax ($Na_2B_4O_7$), 15 rats; *potassium control*, basal diet plus 0.85 per cent potassium chloride, 3 rats; *potassium control boron added*, basal diet plus 0.85 per cent potassium chloride plus 1.0 per cent borax, 6 animals.

The animals were placed in screen-bottomed cages. Food and water were furnished *ad libitum*. Microscopic studies were made of at least two blocks of heart muscle and one of the entire kidney in each animal.

Results. A. *Potassium-deficient group*. These animals on the basal diet (less than 0.01 per cent potassium) grew poorly and all were dead by the fourth week. The average gain was 1.5 gram in the first week and 4.5 during the second week. All showed necrotic foci in the myocardium and changes in the tubular epithelium of the kidneys similar to those we have described elsewhere (6).

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[2] Furnished through the courtesy of Dr. D. F. Robertson, Merck and Company.

B. *Potassium-deficient boron added group.* The rats on the basal diet with added boron (0.22 per cent) grew just as poorly as did those of the above series; there was no prolongation of survival time. The average gain in weight was 1.8 gram for the first week and 4.2 grams during the second week. All showed lesions in the myocardium as well as characteristic renal involvement. When sections of heart and kidney from animals in this and the preceding group were compared, no differences were apparent.

C. *Potassium added group.* Those animals receiving the basal diet supplemented with 0.44 per cent potassium grew well, gaining 12 grams the first week and 14 grams the second. When sacrificed the hearts and kidneys were entirely normal.

D. *Potassium added boron added group.* The 6 rats in this group which received 0.44 per cent potassium and 0.22 per cent boron all grew well and in a comparable manner to the above rats. When killed the hearts and kidneys showed no lesions.

Discussion. As is well known, boron is an indispensable element for the growth of certain plants (7). In contrast, when the rôle of this element in animal nutrition was pointedly studied, no evidence for its indispensability could be found (8–10). The observations, reported by Skinner and McHargue (4) in rats, on the addition of boron to a potassium-deficient diet are of great interest. This experiment was prompted by studies which had been carried out in plants (11) in which potassium deficiency was shown to affect the absorption of boron from synthetic media. Skinner and McHargue (4) are careful to make the point that their experiments provide no evidence for any close interrelationship between boron and potassium in the animal body, the inference being that the effects which they found: increased survival time and greater concentration of hepatic glycogen and body in fat in the boron supplemented rats, are the correction of a boron deficiency.

The morphological observations cited above do not provide any evidence for an interrelation between boron and potassium. Lesions characteristic of potassium deficiency develop as readily in the heart and kidney whether or not boron is added to the diet. Nor were we able to provide any evidence that the growth rate or survival times of our potassium-deficient animals are at all affected by boron supplements. The discrepancies between the two series of experiments may be explained by differences in the potassium and boron contents of the diet; the former being lower in the present experiments, while the latter was about twice as high.

SUMMARY

Morphological studies have been carried out on the heart and kidneys of rats placed on low potassium diets, supplemented with boron. The lesions which occurred in such animals were no different from those encountered in uncomplicated potassium deficiency. Boron supplements have no appreciable effect on growth or survival time under the conditions of this experiment.

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AN EXAMINATION OF SOME FACTORS WHICH ALTER GLOMERULAR ACTIVITY IN THE RABBIT KIDNEY[1]

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These studies on kidney function in the rabbit were undertaken with the two-fold objective of first, establishing such control conditions that this animal could be made available for routine experiments designed to test the effects of various chemical and physical agents on renal activity, and secondly, examining some of the factors which might contribute to alterations in glomerular activity.

Marked alterations in glomerular activity are regularly encountered in cold-blooded animals (1–3). Among mammals the seal exhibits considerable variability of glomerular function with augmentation induced by protein feeding (4), and diminution by asphyxia (5). In the dog the glomerular filtration rate is less labile but varies from day to day with dietary intake of protein and the state of hydration of the animal. Marked changes immediately follow the intravenous infusion of glycin (6). In man there is remarkable constancy in the rate of glomerular filtration even when alterations in renal plasma flow are induced as with pyrogens and adrenalin (7). However, reduction of glomerular filtration rate is acheived with the sustained upright position which presumably elicits a neurogenic vasoconstriction of the afferent arterioles (8).

In the rabbit it has been shown by Kaplan and Smith (9) that creatinine and inulin clearances, which measure the glomerular filtration rate in this animal, increase with increasing urine flows and fail to reach maximal values. Similarly Mayrs and Watt (10) showed that renal blood flow measured directly, and glomerular filtration rate calculated from sulfate clearances, were subject to great variations. Recently Dicker and Heller demonstrated that in the rabbit, unlike the rat, inulin clearances increased with increases in urine flow (11). On the other hand, Walker, Schmidt, Elsom and Johnson concluded after measuring renal blood flows in the rabbit by stromuhr that, although the blood flow varied, there was no correlation between urine flow and the renal blood flow or creatinine clearance (12). White concluded, on the basis of observations made following renal perfusion of a carbon suspension, that deprivation of food and

[1] Supported in part by a grant from the Penrose Fund of the American Philosophical Society.

water did not close glomeruli and that, except possibly in extreme conditions, all the glomeruli in the rabbit were open all the time (13). W. W. Smith failed to find a correlation between urine volume and filtration rate but showed that when the renal blood flow, calculated as the diodrast clearance, increased following administration of mannitol by intravenous infusion there was a concomitant increase in filtration rate as measured by the inulin clearance (14).

Methods. In these experiments rates of glomerular filtration were measured as creatinine clearances (8), renal plasma flows as p-aminohippurate or diodrast clearances (14–16), and variations in the number of functioning glomeruli as the amount of glucose reabsorbed by the tubules under such plasma loads of the sugar as were sufficient to saturate the tubular glucose reabsorptive mechanism (8, 17). Methods used for the precipitation of plasma proteins and for the chemical determination of diodrast and p-aminohippurate were the same as those used by Smith et al. (16). Creatinine was determined by a photoelectric modification of the alkaline picrate method (18), and glucose by the modification of Folin's method described by Shannon, Farber and Troast (17).

In the 46 experiments reported in this paper 368 clearance periods were measured on 41 different male rabbits which ranged in weight from 2.2 to 4 kgm. While the rabbits were in the laboratory they were maintained simply on water and a single complete feed especially prepared for rabbits (Allied Mills, Inc.). Twenty-four to forty-eight hours before an experiment was begun hydration was achieved by withdrawing dry food and allowing the animals to eat all the greens (cabbage usually) which they would take. During the experiment they were restrained in a supine position on an animal board. The male rabbits were catheterized with a lubricated no. 18 or 20 soft rubber 2-holed catheter. It was inserted until the tip projected about 2 inches beyond the pelvic girdle. The bladder was then allowed to contract against the tip which automatically adjusted its insertion until it no longer caused tension of the bladder wall. The rabbit's bladder has sufficient tone to contract down until it snugly wraps the tip of the catheter in such manner that no appreciable amount of urine pools in the bladder. This was repeatedly checked by rinsing the bladder with measured quantities of water and by directly observing the bladder when the body cavity was opened. This proved important because it usually obviated the necessity of washing the bladder after each urine collection. A hole was put in that part of the catheter which projected outside the animal. This allowed the urine to drain readily from it and decreased the dead space in catheter, bladder, ureter and kidney to about 2 ml. The catheter was not tied in place during the experiment. After it had been automatically adjusted by contraction of the bladder wall, it characteristically adhered to the tissues of the urinary passage sufficiently to withstand the animal's struggling without being expelled. While the technique of catheterization was being developed occasionally the catheter would unavoidably enter the large seminal vesicles instead of the bladder. This proved troublesome but rarely occurred after experience with the technique was acquired. Rabbits kept on a diet of dry food characteristically had very concentrated urine with considerable cream-colored precipitates, even though

water for drinking was freely accessible. After 24 hours on a diet of greens, however, the urine was dilute, free of precipitates, and almost colorless.

Blood samples were taken from either the marginal ear vein or the central ear artery on the side not being infused. The ear was customarily heated to dilate the vessels and a 5 ml. blood sample was obtained by syringe. Heparin was used as an anticoagulant and blood was immediately centrifuged following withdrawal. Two milliliters of plasma were used for the chemical determinations, a 15:1 dilution being obtained in the process of precipitating proteins with the cadmium technique. Hematocrits were determined by centrifugation and specific gravity of plasma by the copper sulfate method (19).

Constant intravenous infusions were given into the marginal ear vein via a mercury gravity pump. Mercury in a 250 ml. leveling bulb or separatory funnel dropped adjustable distances through rubber tubing tipped by a fine capillary into a 250 ml. side arm suction flask which contained the infusion fluid. The capillary tip was made from glass capillary tubing which was drawn out and fused until it delivered about 0.5 ml. of mercury per minute under a convenient head of pressure. Tips of this sort were made which steadily delivered as little as 0.1 ml. per minute. Transparent plastic tubing led away from the side arm of the suction flask to the infusion needle. Inserted in the system several inches beyond the needle was a length of two inches of good gum rubber tubing which would not leak after being repeatedly punctured in the process of administering the priming solution. A no. 19 medium bevel hypodermic needle one-half inch long was used for the intravenous infusion, and was made to fit into the tubing by sawing and filing until its attachment was reduced to an appropriate size to fit without leaking. To start delivery of the infusion fluid a clamp on the vertical rubber tubing between the side arm flask and the leveling bulb was removed. This allowed the mercury to run through the calibrated glass capillary tip displacing infusion fluid which in turn was forced out through the side arm. Transparent tubing was helpful here to detect air bubbles which might enter the venous system. With the infusion fluid flowing through the needle it was inserted into the marginal ear vein and secured with a paper clip. Tubing of small diameter permitted considerable movement of the animal's head without disturbing the needle within the vein. The tube ran through a warm water bath of such temperature that the fluid was delivered into the vein at about the body temperature of the rabbit.

The object of infusion was to introduce creatinine, p-aminohippurate and glucose into the circulating system at the same rate these compounds were being excreted so that their concentrations in the plasma would remain relatively constant during the experiment. They were originally introduced into the circulatory system in a priming injection that attained the desired plasma level of each compound. Regulating the rate of delivery and concentration of the infusion fluid served to maintain these plasma levels. The priming injections were administered by syringe needle through the gum rubber insert in the infusion delivery tube. A typical control rabbit (R_{54}) which weighed 2.9 kgm., and whose kidneys weighed 14.5 grams, was infused at the rate of 0.6 ml. per minute with a

solution containing 10 grams of mannitol, 3.2 ml. of a 20 per cent solution of Na p-aminohippurate, 3 grams of creatinine, and 40 grams of glucose made up to a volume of 300 ml. with Ringer's solution. The priming injection consisted of 1 gram of mannitol, 0.1 ml. of 20 per cent Na p-aminohippurate solution, 250 mgm. of creatinine, and 2.5 grams of glucose. The first blood collection was taken one hour later and its plasma concentrations were as follows: 1.68 mgm. per cent of p-aminohippurate, 30.5 mgm. per cent of creatinine and 955 mgm. per cent of glucose. The blood collections were taken from the ear artery during the experiment. Over a period of 3.5 hours the p-aminohippurate plasma concentration rose from 1.68 to 1.70 mgm. per cent, creatinine from 30.5 to 37.2 mgm. per cent and glucose fell from 955 to 793 mgm. per cent. The urine flows varied between 1.2 and 0.74 ml. per minute.

An important factor in assuring accuracy with renal clearance determinations is to maintain the urine flows high enough that a reliable measurement of their volume can be made. Infusion with saline solution was not enough to maintain adequate urine flows in the rabbit. Hydration by administration of water by stomach tube resulted in elevating the rates of glomerular filtration and renal plasma flow so that steady control values could not be attained. Hydration was achieved by placing the animals on a cabbage and lettuce diet for 24 or more hours before the experiment began. The rabbits ate large quantities of these greens and a state of hydration was achieved which was not accompanied by fluctuating glomerular filtration rates or renal plasma flows. Another important factor in maintaining high urine flows during the experiment was the inclusion of the osmotic diuretic mannitol in both the infusion fluid and the priming injection.

Urine was collected and measured directly in a small graduated clyinder. All samples were diluted to 2 ml. per minute to obviate difficulties in chemical determination due to fluctuations in rates of tubular water reabsorption. A 2 ml. sample of this was subsequently diluted 125:1 in a volumetric flask. Two milliliter aliquots of this diluted urine were used for the determination of the glucose concentration, 10 ml. for the creatinine, and 2 ml. for the p-aminohippurate determination. Ten milliliter aliquots of the diluted plasma filtrate were used for the p-aminohippurate determination, 5 ml. for creatinine and 2 ml. of a 90:1 dilution for the glucose determination.

RESULTS. A. *Basal values and the absence of any effect of the animal's position during the experiment on systemic blood pressure or renal function values.* Rabbits restrained in a supine position on an animal board for as long as 5 hours maintained constant blood pressure and renal clearance values. To test the possibility that different values might be obtained with the animal in some position other than supine, renal blood flow and glomerular filtration rates were measured and blood pressure determined with the rabbits maintained in prone and in sitting positions as well. Such experiments disclosed that no alteration of these factors was obtained with changing positions.

Control rabbits in these experiments had body weights ranging from 2.2 to 4 kgm. and kidney weights from 14.5 to 24 grams. Kidney and body weights did not bear a close relation to one another, and also there was not a close correlation

between either of these and renal clearance values. Hence, in these experiments the infusion rate and the concentrations of creatinine, glucose, and p-aminohippurate in the infusion fluid and priming solution were administered without variation from animal to animal despite differences in body weight. In figure 1 is represented the renal clearance values characteristically obtained in a control animal. This experiment (R54) was outlined in detail under "methods". No significant shifts in renal plasma flow or glomerular filtration rates were obtained in over 3 hours.

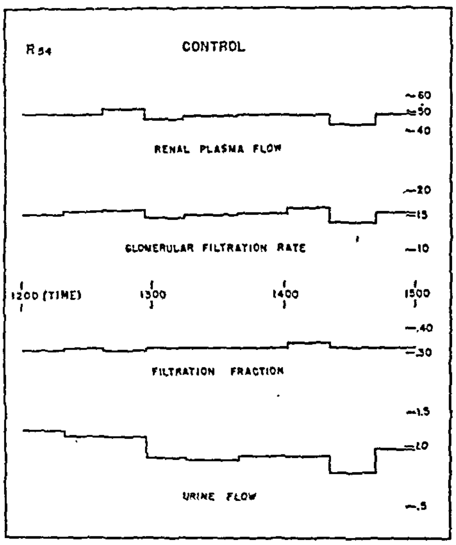

Fig. 1

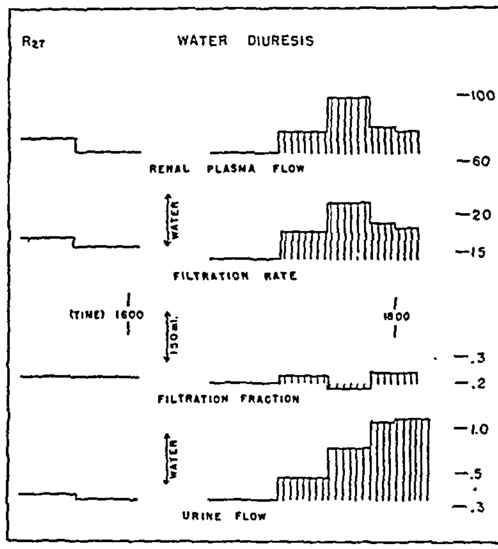

Fig. 2

Fig. 1. Control. An experiment showing characteristic control values for the renal plasma flow, the glomerular filtration rate, the filtration fraction and the urine flow in ml./min. in a rabbit which weighed 2.9 kgm. and whose kidneys weighed 14.5 grams. These values remainedconstant for the three hours during the experiment while the rabbit was restrained in a supine position on an animal board.

Fig. 2. Water diuresis. This rabbit responded to the administration of 150 ml. of warm water with a three-fold increase in urine flow which was accompanied by concomitant increases in the renal plasma flow and the glomerular filtration rate. Renal plasma flow, glomerular filtration rates and urine flows are expressed in terms of milliliters per minute.

In several experiments the effects of altering the rate of infusion were studied by augmenting the usual infusion flow with an equivalent amount of Ringer's solution. Doubling the rate of infusion in this manner had no effect on the creatinine and p-aminohippurate clearances.

B. *Recruitment of glomerular activity induced by the administration of water by stomach tube.* Rabbits kept in the laboratory on a diet of dry food maintain very low urine flows even though free access to drinking water is provided. In the course of these renal clearance determinations it was soon found that infusing Ringer's solution intravenously did not ensure urine flows sufficiently high to be measured accurately. Diuresis was attempted by administering tepid water

by stomach tube. This was found to be generally effective in increasing urine flows but was accompanied by such corresponding increases in the creatinine and p-aminohippurate clearances that it was impossible to obtain steady control values for experiments designed to test factors which might alter renal activity.

Figure 2 illustrates the effect of the administration of water by mouth on the renal plasma flow and filtration rate. The urine flow increased from 0.3 ml. per minute to over 1.0 ml. per minute following the administration of 150 ml. of water to this rabbit which weighed 3 kgm. This was accompanied by an increase in the renal plasma flow from the average control value of 70 ml. per minute to 104, while the glomerular filtration rate rose from 14.3 ml. per minute to 20.2. The filtration fraction remained unchanged.

To test whether this increase in glomerular activity was due to previously non-functioning glomeruli becoming active under the effects of this increased water load, the rate of tubular reabsorption of glucose was calculated with the glucose plasma level raised sufficiently high to ensure saturation of the tubular glucose reabsorptive mechanism. In figure 3 the glucose Tm is shown to parallel the increase in renal plasma flow and glomerular filtration rate. In this animal which weighed 2.2 kgm. the administration of 110 ml. of warm water by stomach tube was followed by an increase in the glucose Tm from 37 mgm. per minute to 64, while the renal plasma flow increased from 34.6 ml. per minute to 63 and the glomerular filtration rate rose from 11.9 ml. per minute to 21.4. This increase in the amount of glucose reabsorbed by the tubules would not parallel increases in the rate of glomerular filtration unless glomeruli previously inactive began actively filtering after the administration of water. The renal plasma flow, the filtration rate and the glucose Tm (number of functioning glomeruli) were found invariably to increase following diuresis obtained by administering water by stomach tube. Diuresis, however, did not invariably follow the administration of water in this manner. The mechanism underlying this increase in glomerular activity following the administration of water is obscure. Hematocrits and the specific gravity of blood were routinely measured but no regular relation between the degree of hemodilution and alterations in glomerular activity was found.

C. *Diuresis without recruitment of glomerular activity following the intravenous administration of mannitol and theophylline.* Although increases in urine flow were accompanied by corresponding changes in renal blood flow and glomerular filtration rate when the diuresis was induced by administering water by stomach tube, variations in urine flow could be obtained in the absence of alterations in glomerular activity. Mannitol and theophylline administered intravenously were both effective agents in increasing urine flow by diminishing the rate of tubular water reabsorption.

Mannitol was found to be a very dependable agent in promoting high urine flows and was regularly incorporated in the infusion fluid for this purpose. In rabbit R52 represented in figure 3, after two control periods an additional 1.5 grams of mannitol was dissolved in several milliliters of Ringer's solution and injected intravenously. This was followed by a doubling of the urine flow without a

corresponding change in either filtration rate or renal plasma flow. In a similar experiment the urine flow rose from 0.14 to 0.64 ml. per minute following the injection of mannitol while the glomerular filtration rate actually was depressed from 46.2 to 37.6 ml. per minute, and the glucose Tm fell from 33.4 to 25.6 mgm. per minute. There can be no doubt that this diuretic effect of mannitol was simply due to alterations in the tubular reabsorption of water.

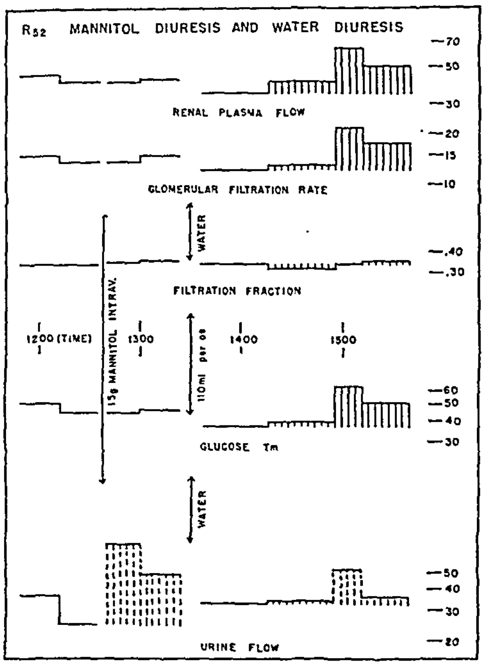

Fig. 3

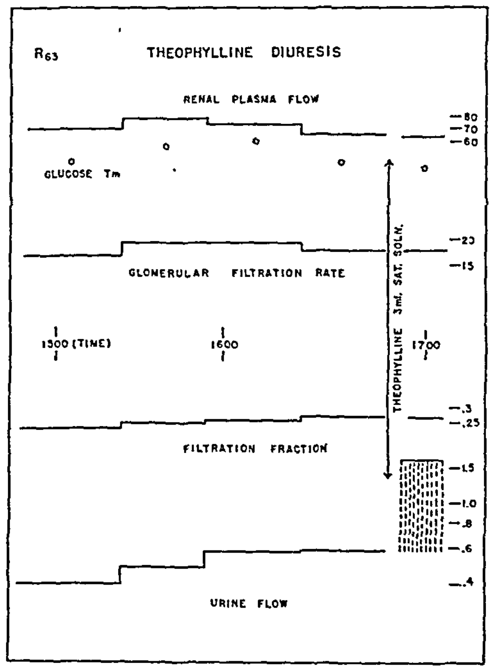

Fig. 4

Fig. 3. Mannitol diuresis and water diuresis. This experiment demonstrates that diuresis can be achieved without accompanying alterations in glomerular activity. Mannitol injected intravenously merely affected tubular water reabsorption and markedly increased the urine flow withoutproducing concomitant alterations in renal plasma flow or glomerular filtration rate. On the other hand, the administration of 110 ml. of water by stomach tube later in the experiment resulted in increased glomerular activity along with an augmented urine flow.

Fig. 4. Theophylline diuresis. The xanthine diuretic, theophylline, effectively increases urine flow by its action in simply blocking tubular reabsorption of water. Diuresis is achieved while the renal plasma flow, the glomerular filtration rate and the number of functioning glomeruli are unaffected following its intravenous injection. Renal plasma flow, glomerular filtration rates and urine flows are expressed intermsof ml./min.

Theophylline administered intravenously in the rabbit had an effect similar to that of mannitol. In figure 4 the urine flow can be seen to rise from 0.6 to 1.65 ml. per minute without a concomitant change in either the glomerular filtration rate or the renal plasma flow following the injection of 3 ml. of a saturated solution of theophylline. Also, in control clearance periods the random variations in urine flow were characteristically independent of simultaneous alterations in the rates of glomerular filtration or renal plasma flows. Hence, though altera-

tions in glomerular activity accompany diuresis following the administration of water by stomach tube, variations in urine flow can also, under other conditions, be induced simply by alterations in the rates of tubular water reabsorption without any corresponding changes in renal plasma flows or glomerular filtration rates.

D. *Reduction of glomerular activity following the administration of ether.* The lability of the afferent circulation previously demonstrated by the administration of water was further shown by the reduction in glomerular activity following the inhalation of ether. In six rabbits examined ether inhalation invariably resulted in a diminution of the renal plasma flow, the glomerular filtration rate, the glucose

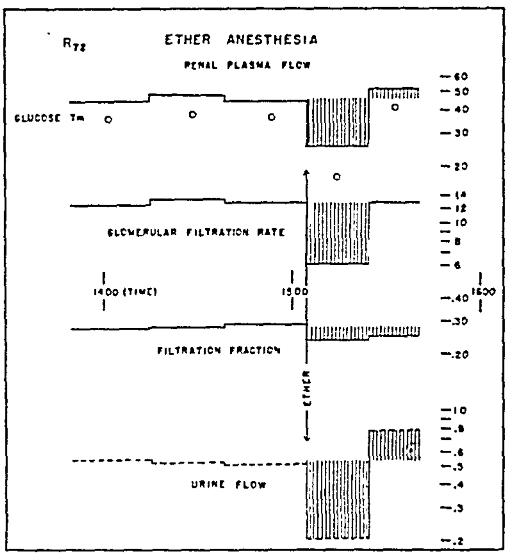

Fig. 5

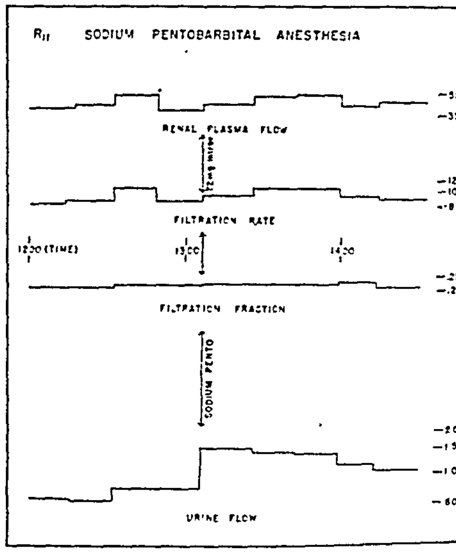

Fig. 6

Fig. 5. Ether anesthesia. The relative lability of glomerular activity in the rabbit is demonstrated by the effectiveness of ether inhalation in reducing renal plasma flow, glomerular filtration rate and number of functioning glomeruli (glucose Tm).

Fig. 6. Sodium pentobarbital anesthesia. This experiment demonstrates that the state of anesthesia per se does not result in a depression of glomerular activity. Renal plasma flow, glomerular filtration rates and urine flows are expressed in terms of ml./min.

Tm, and the urine flow. These reductions in renal function were obtained without significant changes in systemic blood pressure. Illustrated in figure 5 is a typical experiment in which, after 3 control periods, ether was administered by inhalation cone for 12 minutes. Despite marked reduction in renal plasma flow and filtration rate the return to normal values was rapid. During the last period with the inhalation cone removed the animal was still sufficiently anesthetized to abolish the corneal and limb reflexes. These results, together with the observations on sodium pentobarbital effects, indicate that this reduction in glomerular activity is not a feature of the state of anesthesia per se, but rather is the result of neurogenic reflex afferent arteriolar vasoconstriction induced by the administration of this irritant anesthetic agent.

E. *Anesthesia without reduction of glomerular activity following the administration of sodium pentobarbital.* Sodium pentobarbital was administered in anesthetic doses to 3 rabbits without affecting the renal hemodynamic values in any way. In figure 6 an experiment is represented in which 72 mgm. of sodium pentobarbital was injected intravenously. Despite the increased urine flow following the administration of the anesthetic there was no concomitant change in renal plasma flow or glomerular filtration rate. The diuretic effect elicited in this experiment was not obtained in the others. These experiments demonstrate that the rabbit can be deeply anesthetized without inducing any changes in glomerular activity.

Discussion. The outstanding feature of renal function in the rabbit is the relative lability of glomerular activity. Marked increases in renal blood flow and glomerular filtration rates can be obtained following the administration of water by mouth and diminutions can be observed following the administration of ether by inhalation. Alterations in the number of functioning glomeruli accompany these changes. This is demonstrated by the corresponding fluctuations in the amount of glucose reabsorbed by the tubules when the plasma glucose concentration is sufficiently high to ensure saturation of those tubules being perfused by the filtrates of actively functioning glomeruli. These observations complement those of Kaplan and Smith (8) who noted that the inulin clearance of rabbits rose with increases in urine flow, and those of Dicker and Heller (10) who further noted that alterations in the rate of tubular excretion of diodone also accompanied these rises. While these fluctuations in glomerular activity are not characteristically a feature of renal activity in man, the rabbit is not distinctive in this respect even among mammals. Pitts (6) has suggested that this variability of glomerular function, also noted in the seal and dog, is perhaps an adjustment to needs precipitated by periodic gorging of food and water in these animals in contrast to those eating and drinking practices of the ancestors of man which might have characteristically been more frequent and regular.

The behavior of the rabbit in response to ether inhalation differs from that observed in the dog (Craig, Visscher and Houck, 20) in that the former responds more sensitively by showing marked reductions in renal blood flow and filtration rate with light anesthesia, whereas no such depression of renal function is elicited in the dog unless the animal is placed under deep anesthesia. In both the rabbit and the dog the renal changes are attributed to neurogenic constriction of the afferent arterioles of the kidney. The absence of an effect of sodium pentobarbital anesthesia on renal function noted here was similarly observed by Corcoran and Page (21).

However, despite the fact that urine flows in the rabbit fluctuate when changes are induced in the rate of glomerular filtration, under basal conditions the filtration rate and renal plasma flow remain constant for long periods of time, and alterations in urine flow then are simply the result of alterations in the rates of tubular water reabsorption. This rôle of the renal tubules in regulating the rate of water elimination in the rabbit can be dramatically demonstrated by the intravenous administration of the diuretic agents, mannitol and theophylline.

When the method of treatment outlined in this paper is followed the rabbit constitutes an excellent animal for renal clearance studies. Even the untrained animal can be restrained for hours and yet maintain uniform blood pressure and renal function values. Rabbits can be kept in good condition in the laboratory for long periods of time on a single complete feed, and they can be adequately hydrated in preparation for renal clearance studies simply by feeding greens for a 24 hour period prior to the experiment. Failure to take into account lability of glomerular activity, especially as it is affected by water administration, has prevented the rabbit from being widely adopted for renal function studies. However, with adequate provisions made to ensure steady control values this animal, because of the ease of handling and because it can be used untrained, is well suited for routine instructional and research studies in kidney physiology.

SUMMARY

Marked alterations in the rate of glomerular filtration, the renal plasma flow, and the number of functioning glomeruli were readily produced in the rabbit. Increases in these values followed the administration of water by stomach tube and decreases accompanied the administration of ether by inhalation.

Under basal conditions, however, the glomerular filtration rate, the renal plasma flow and the number of functioning glomeruli remained constant for long periods of time. Random variations in urine flow under control conditions were simply the result of alterations in the rates of water reabsorption by the renal tubules. Similarly diuresis following the intravenous administration of mannitol and theophylline was acheived by reduction of tubular water reabsorption without alterations in glomerular activity.

Deep anesthesia was induced by the intravenous administration of sodium pentobarbital without the occurrence of concomitant changes in blood pressure or renal function values.

The uniformity of renal blood flow and glomerular filtration rates which were characteristically maintained under control conditions for long periods of time even in the untrained rabbit recommends the use of this animal for instructional and research studies in kidney physiology.

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EFFECT OF EXPERIMENTAL NEUROGENIC HYPERTENSION ON RENAL BLOOD FLOW AND GLOMERULAR FILTRATION RATES IN INTACT DENERVATED KIDNEYS OF UNANESTHETIZED RABBITS WITH ADRENAL GLANDS DEMEDULLATED[1]

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This study was undertaken to examine responses made by the renal circulation to increases in systemic blood pressure in unanesthetized animals. The kidneys were dénervated and the adrenal glands demedullated to avoid such nervous or hormonal effects as might be reflexly induced by the procedure employed in neurogenically obtaining pressure increases via severing the depressor nerves and clamping the carotid arteries. Interest in this problem stems not only from the general importance of an understanding of basic pressure-flow relationships, but also from the attention directed to alterations in renal blood flow as an explanation of the possible rôle of the kidney in the genesis of hypertensive disease.

It has been repeatedly shown that the most characteristic feature of the renal circulation is the constancy of renal blood flow which is maintained despite extensive variations in blood pressure. This was demonstrated in a classic experiment by Burton-Opitz and Lucas in 1911 (1). Renal blood flow was measured by stromuhr on the renal vein in dogs narcotized with chloroform or ether, and rises in arterial blood pressure were obtained by central excitation of the vagi. Characteristically no change in blood flow was detected despite pronounced rises in arterial blood pressure. This led them to conclude that the renal blood vessels tonically retain the normal size of the bloodbed. After renal denervation, in this preparation, the accommodation in increased pressure was lost and the renal blood flow was readily altered passively. However, Opitz and Smyth showed in dogs narcotized with morphine-pernactone that blood pressure variations elicited by the carotid sinus reflex did not alter renal blood flow as measured by stromuhr in intact or in denervated kidneys (2). The early literature dealing with the effect of changing pressure on renal blood flow as estimated by oncometer and stromuhr in anesthetized animals has been reviewed by Smith (3).

An opportunity to measure renal blood flow and glomerular filtration rates in unanesthetized animals was provided with the introduction of clearance techniques. Bing, Thomas and Waples (4) induced chronic hypertension neurogenically in dogs, and demonstrated that the renal blood flow and glomerular filtration rates remained constant or fell with the onset of increased blood pressures. The studies on the unanesthetized rabbit reported in this paper indicate

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[2] Died August 7, 1946.

that this adjustment to acute pressure increases is made without the mediation of reflex nerve action or secretory activity of the adrenal medulla.

METHODS. Renal clearance procedures employed in this study were discussed in detail in an earlier paper (5). Renal plasma flow was measured as the p-aminohippurate clearance. The glomerular filtration rate was represented by the creatinine clearance, and estimation of the number of functioning glomeruli was obtained by calculation of the amount of glucose reabsorbed by the renal tubules when the glucose plasma level was high enough to ensure saturation of those tubules being perfused by glomerular filtrate.

The male rabbits used in this study varied in weight between 2.2 and 3.8 kgm. At least 3 weeks before the acute experiment the kidneys were denervated by completely stripping the renal pedicles and the adrenal glands were demedullated.

Blood pressure was measured directly by cannulation of the femoral artery. Under local anesthesia the depressor nerves were severed and, following several control periods, elevated systemic blood pressure was induced by clamping the carotid arteries. Blood and urine samples were collected before and after elevation of blood pressure and chemical analyses were made for creatinine, glucose, and p-aminohippurate in each. Blood hematocrits were recorded and specific gravity of blood samples determined by the copper sulfate solution method.

Measurement of retraction of the nictitating membrane of the eye was used on 3 animals to test the possibility that detectable amounts of adrenine were liberated by the experimental procedure used to raise blood pressure. To test the possibility that denervation was not complete and that renal response to the neurogenic hypertensive procedure might be reflex, the vagus nerve was stimulated electrically to keep the blood pressure normal while the carotid arteries were clamped in 3 animals with adrenals demedullated and kidneys denervated.

RESULTS. The effects of increased blood pressure, neurogenically induced, were measured on the kidneys of 10 male rabbits which previously had their kidneys denervated and adrenal glands demedullated. Figure 1 illustrates an experiment in which, after 3 control periods of 21, 26, and 15 minutes each, the mean systemic blood pressure was raised from 75 to 110 mm. Hg by clamping the carotid arteries following section of the depressor nerves. This elevated pressure was maintained for 9 minutes. During the last 17 minute period the carotid circulation was restored by removing the clamps. The 47 per cent increase in pressure resulting from clamping the carotids did not significantly alter renal plasma flow (p-aminohippurate clearance), or glomerular filtration rate (creatinine clearance). In the period before clamping the carotid arteries the renal plasma flow was 72 ml. per minute and the glomerular filtration rate was 23.9. After the blood pressure was raised these values were 73 and 24.8 ml. per minute respectively. The number of functioning glomeruli (glucose Tm) was actually diminished with the elevation in pressure. Forty-seven and nine-tenths milligrams per minute of glucose were reabsorbed by the tubules in the period with the pressure at 75 mm. Hg, and 45.9 mgm. per minute at the elevated pressure.

Figure 2 represents the effect of elevated blood pressure on renal plasma flow in all the animals studied. The mean control systemic pressure in the femoral artery was 79 mm. Hg, and the mean increase following clamping the carotid arteries was 34 mm. Hg. Renal plasma flow increased slightly in 6 experiments and actually decreased in 3. A mean pressure increase of 43 per cent resulted in such accommodation by these denervated kidneys that the mean plasma flow increase was only 5 per cent.

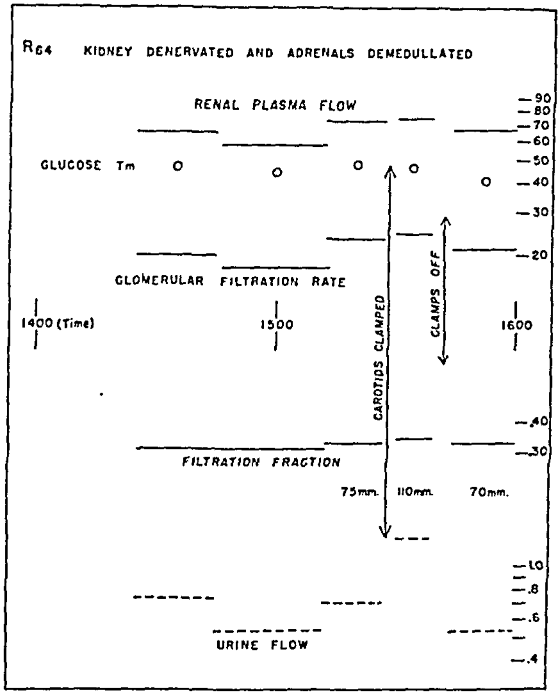

Fig. 1. Renal response to the elevation of systemic blood pressure in a large male rabbit (R$_{64}$, 3.8 kgm.) whose kidneys were denervated and adrenal glands demedullated 3 weeks before the acute experiment. After 3 control periods during which the mean pressure, *with depressor nerves cut, was 77, 76 and 75 mm. Hg respectively, the pressure was suddenly* elevated to 110 mm. Hg by clamping the carotid arteries. This 47 per cent increase in pressure did not significantly affect the renal plasma flow (p-aminohippurate clearance), the glomerular filtration rate (creatinine clearance) or the filtration fraction. The elevation in urine flow is characteristic and was noted even in those experiments where an actual diminution in renal plasma flow and glomerular filtration rate was induced with increased blood pressure. Renal plasma flow, glomerular filtration rate and urine flow are expressed as ml./min.

The effect of increased systemic blood pressure on glomerular filtration rate is illustrated in figure 3. A rise in filtration rate accompanied the increase in pressure in every experiment but one, but, as with renal plasma flow, the slight increase in rate indicated that an adjustment to pressure rise was made in these denervated kidneys which prevented the filtration rate from passively following the pressure increase. The 43 per cent mean systemic pressure increase resulted in raising the mean glomerular filtration rate only 8 per cent. The number of functioning glomeruli paralleled, in general, the glomerular filtration rate.

When Lamport's formulas were used to calculate renal afferent and efferent arteriolar resistance (6) it became apparent that the adjustment made by the kidney to pressure rises was chiefly an increase in resistance, presumably vasoconstriction, afferent to the glomeruli. Figure 4 illustrates the invariable increase in afferent resistance which accompanied increased systolic pressure, whereas no such uniform change in efferent resistance was noted.

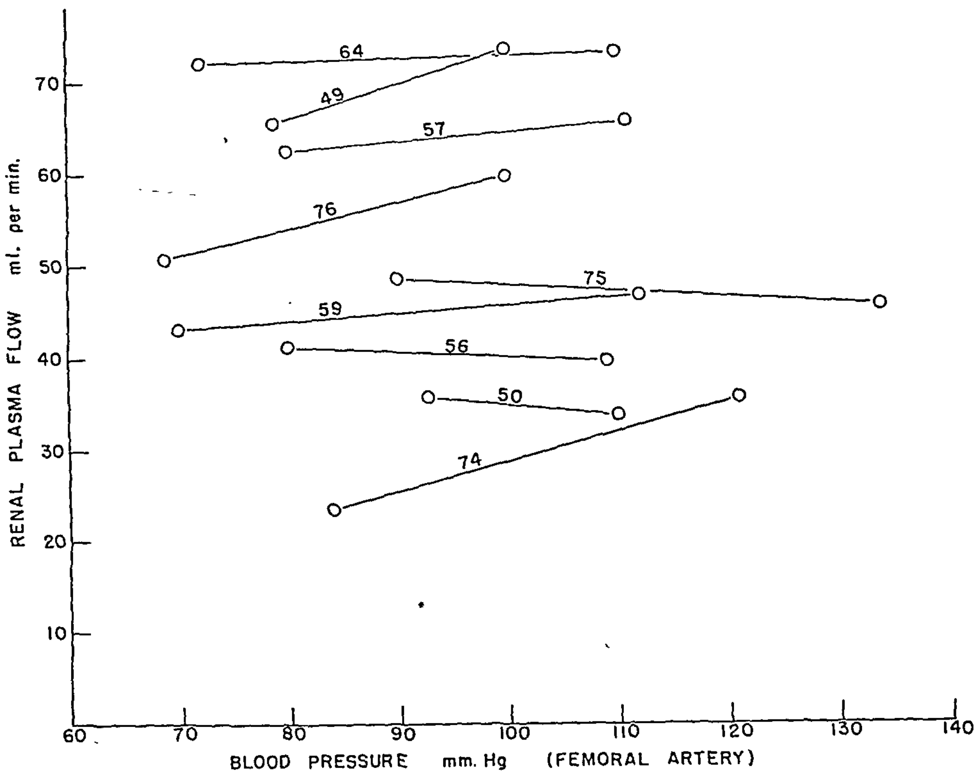

Fig. 2. The effect of elevated blood pressure on renal plasma flow in all animals studied. Each line represents a separate experiment and connects plasma flow in control period prior to clamping carotid arteries with flow values after elevated pressure was induced. Numbers identify individual animals. In 3 instances the renal plasma flow was actually less at the higher pressure than during the control period. Animal 74 had one kidney. All kidneys were denervated and adrenal glands demedullated.

Experiments were designed which demonstrated that the adjustment made by the kidney to pressure changes were not due, first, to incomplete denervation of the kidneys and, secondly, to reflex secretion of adrenine induced by the experimental procedure employed in elevating blood pressure. In 3 rabbits with adrenals demedullated and kidneys presumably denervated the carotids were clamped following section of the depressor nerves, but blood pressure was prevented from rising by electrical stimulation of the vagus nerve. This did not induce increases in renal afferent resistance in any of the animals. The possible liberation of adrenine following neurogenic pressure increase was tested in 3 other animals with adrenal glands demedullated and kidneys denervated. Retraction

of the nictitating membrane of the eye was used to test for the release of adrenine into the circulatory system. In 2 animals no retraction accompanied the procedure and in one rabbit a slight retraction was recorded. This indicated that it was quite unlikely that in these animals with adrenals demedullated the increase in renal afferent resistance was due to adrenine reflexly liberated from an extra-adrenal source.

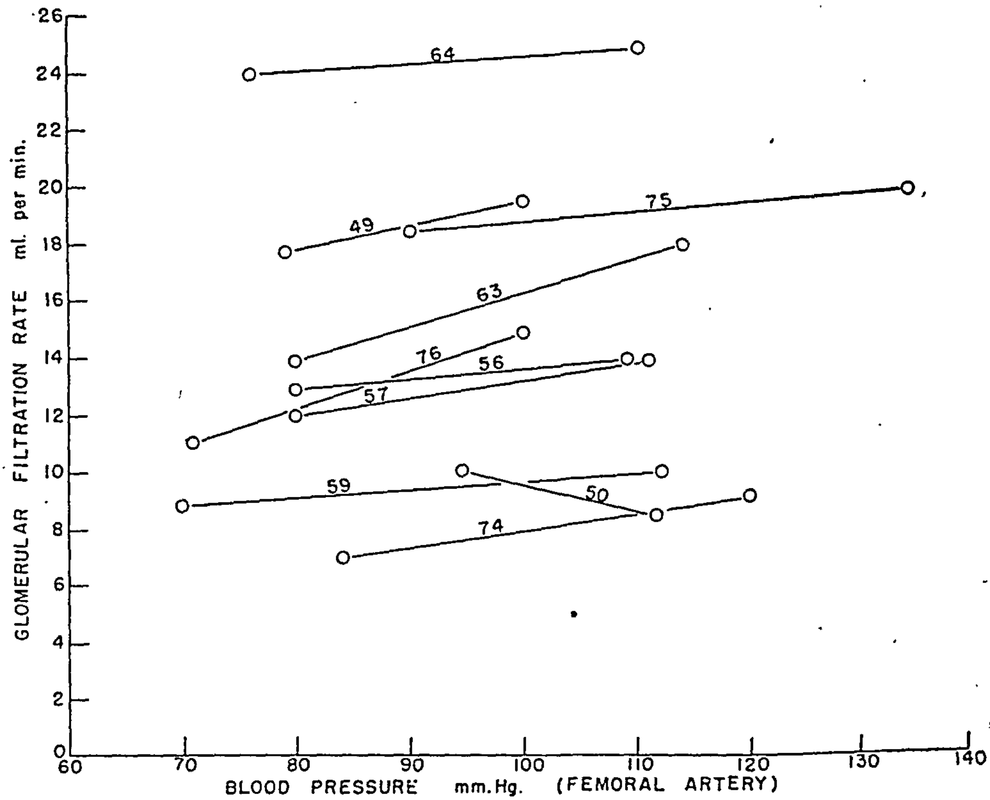

Fig. 3. The effect of elevated blood pressure on glomerular filtration rates in all rabbits examined. In all experiments but one (R_{50}) the glomerular filtration rate rose with increased pressure but the rates did not passively follow pressure rises. The mean pressure increase was 43 per cent whereas the glomerular filtration rate increased only 8 per cent Animal 74 had one kidney. All kidneys were denervated and adrenal glands demedullated.

Discussion. The experiments just described illustrate the autonomy of the renal circulation in the unanesthetized animal, particularly with reference to increases in systemic blood pressure. This relative constancy of renal blood flow at normal or high blood pressures has repeatedly been demonstrated (3, 4, 7–9). The observations here on the rabbit further demonstrate, however, that accommodation to pressure increases is obtained in totally denervated kidneys and without the mediation of adrenine secretion.

Winton (9), Lamport (10) and Selkurt (7) have pointed out that as a consequence of filtration at the glomerulus post-glomerular blood undergoes hemoconcentration and increased viscosity until it is subsequently diluted by tubular

water reabsorption. Should the glomerular filtration rate vary with blood pressure, corresponding alterations in viscosity would result in buffering the renal circulation against pressure fluctuations with the result that constant renal blood flow would be maintained. This explanation cannot be applied to the experiments in this study because the glomerular filtration rate, as well as the renal plasma flow, remained relatively constant despite marked increases in pressure. No increase in blood viscosity could be obtained which would be great enough to counteract the increased pressure produced. The failure of the filtration rate

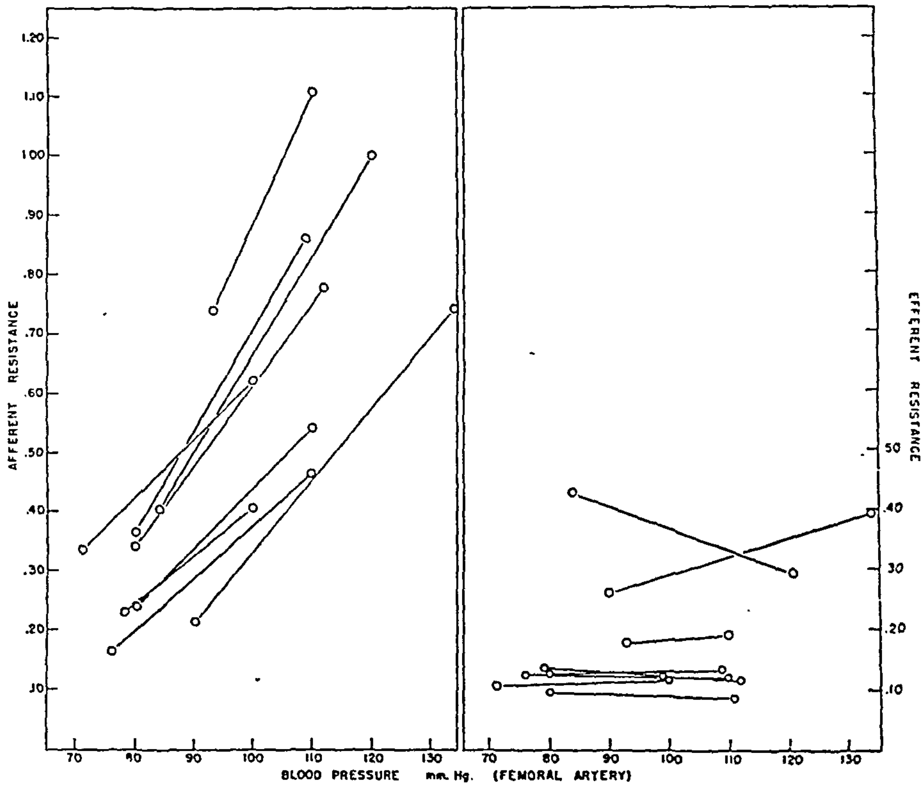

Fig. 4. The effect of increased blood pressure on resistance in the renal circulation afferent and efferent to the glomeruli as calculated by Lamport's formulas. These results are from all animals studied and relate to the same data presented in figures 2 and 3. The increase in afferent resistance is achieved in these denervated kidneys presumably without the mediation of adrenine.

passively to follow blood pressure rises indicates that the accommodation in the renal circulation which resulted in uniform flow was made afferent to the glomeruli. The basic mechanism by means of which the renal circulation accommodates to elevations in blood pressure under these circumstances remains obscure.

SUMMARY

The relative autonomy of the renal circulation in unanesthetized rabbits, particularly with reference to increases in blood pressure, persists even in animals with kidneys denervated and adrenal glands demedullated.

Acute neurogenic blood pressure elevations were experimentally induced and clearance techniques employed to measure the effect of pressure increases on renal plasma flow and glomerular filtration rates in 10 male rabbits. A mean pressure rise of 43 per cent for all animals studied was accompanied by an increase in renal plasma flow of 5 per cent, and in glomerular filtration rate of 8 per cent. Three of the animals showed decreased renal plasma flow with the increase in blood pressure, and a fall in glomerular filtration rate was noted in one.

A rise in resistance afferent to the glomeruli accompanies blood pressure elevation. The basic mechanism accountable for this pressure-flow accommodation remains obscure.

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EFFECT OF ETHER AND PENTOBARBITAL ON THE POLARISATION STATE OF CENTRAL NERVOUS ELEMENTS

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Koch (1927) found that ether and chloroform decrease the injury potential of peripheral nerve. Höber, Andersh, Höber and Nebel (1939) observed depolarisation in frog and crab nerve when treated with the narcotics ethyl urethane, and chloral hydrate and with amyl alcohol, and in crab nerve with the local anesthetics cocaine and novocaine. Bishop (1932) had previously reported, however, that amyl alcohol and cocaine in concentrations sufficient to abolish nerve conduction produce a "positive resting potential". This was recently confirmed by Bennet and Chinburg (1946) for a number of local anesthetics. However, they found that some of these drugs when permitted to act over longer periods will cause nerve depolarisation. Wright (1947) showed that ether and alcohol vapor cause a distinct depolarisation of mammalian nerve, which to a certain degree parallels the depression of the action potential caused by these agents.

Since the narcotics seem to affect the polarisation state of the peripheral nerve, it was of interest to investigate the effect of such drugs on the polarisation of central nervous elements. It has been found (van Harreveld, 1946) that during asphyxiation of the spinal cord it is possible to lead off from the gray matter a "depolarisation potential", the course of which indicates that first the more sensitive parts of the neuron (probably mainly the nerve cells) depolarise, followed by the depolarisation of the more resistant parts (fibers). The methods used in that investigation have been found to be feasible for the examination of the effects of drugs on the polarisation state of central nervous elements. The action of two narcotics, ether and pentobarbital (nembutal), will be described.

Methods. An active electrode was placed into the gray matter of the spinal cord at the level of L 7 or S 1, and an indifferent electrode on an anterior root of one of these segments. After amplification the potential changes were recorded with a coil type galvanometer (for a more detailed description see van Harreveld, 1946).

Curare (Intocostrin, E. R. Squibb & Sons, 0.5 cc/kgm.) was administered to prevent disturbance of the electrodes by active movements of the animal. Artificial respiration was given during the entire experiment. The blood pressure was recorded continuously. It was usually possible to maintain a blood pressure of at least 10 cm. of mercury by infusing, when necessary, adrenaline (10^{-5}) into the jugular vein. The maintenance of a reasonable blood pressure is an essential part of these experiments since the narcotics, especially in large doses, tend to cause a drop in blood pressure. When sufficiently pronounced this impairs the oxygenation of the cord and thus causes depolarisation. It has been possible to inject with this technique up to 5 times the narcotic dose

of pentobarbital, 40–50 mgm./kgm. (Fulton and Keller, 1932), without a marked drop in blood pressure.

Pentobarbital was injected into the carotid artery. Larger amounts can be administered by this method at a higher rate, without endangering the heart action, than when this drug is given intravenously. It was customary to add a small amount of adrenaline to the pentobarbital to combat the drop in blood pressure which would otherwise result from such an injection.

Ether was administered by bubbling the air for the artificial respiration through an ether bottle.

Asphyxiation of the cord was produced by clamping the aorta between the diaphragm and the coeliac artery. The blood supply through the spinal arteries had been arrested by cutting the spinal cord at Th 11 or 12.

Cats were used exclusively. The preparation was made in ether narcosis.

RESULTS. *The effect of pentobarbital on the polarisation state of the spinal cord.* The effect of pentobarbital was investigated in preparations in which asphyxiation of the cord had been shown to produce the usual depolarisation potential. As described before (van Harreveld, 1946) clamping of the aorta causes a galvanometer deflection indicating a developing negativity of the gray matter. This deflection usually reaches a maximum in 1.5 to 2.5 minutes. The amplification selected for the present experiments was such that this maximum caused a 3 to 5 cm. galvanometer deflection, representing a potential difference varying from a few to as high as 20 mV.

After having thus established the response of the spinal cord to asphyxiation, pentobarbital was injected into the carotid artery in doses of 50 mgm./kgm. (the narcotic dose). The administration of such a large dose of pentobarbital in the course of 1 to 2 minutes usually has surprisingly little effect. In many experiments no deflection at all could be ascribed to the injection of the drug; in others it caused a small galvanometer movement, the direction of which was inconsistent. This movement was sometimes temporary, the galvanometer returning to its original position a few minutes later. The subsequent injection of several such doses of pentobarbital had no more effect than the first, as long as it was possible to prevent a marked drop of the blood pressure by the infusion of adrenaline.

The absence of a consistent electrical effect might indicate that the administration of pentobarbital does not affect the polarisation state of the spinal cord. However, with the method used, depolarisation can be detected only if part of the neuron depolarises, as for example during the first phases of cord asphyxiation, when the cell bodies depolarise but the fibers are still little affected (van Harreveld, 1946). If the entire neuron depolarises uniformly, no potential difference can be expected between the gray and white matter of the cord. The observation that pentobarbital does not cause a consistent potential difference thus may also indicate a uniform change in the polarisation state of the entire neuron. To distinguish between these possibilities, use has been made of the depolarisation potential during short asphyxiations of the cord, as a measure of the polarisation state of the neuron parts most sensitive to oxygen lack (cells).

If pentobarbital depolarises the neuron one would expect a decrease of the asphyxial depolarisation potential.

It was found that the deflection caused by clamping the aorta grew smaller and smaller as more and more pentobarbital was administered. In table 1 the data of 5 experiments are collected. The aorta was clamped for a period long enough to record the maximum deflection (1.5 to 2.5 min.). After establishing the value of the prenarcotic deflection, a dose of 50 mgm. of pentobarbital per kgm. bodyweight was injected, and 6 to 7 minutes later its effect on the asphyxial deflection was examined. The injection of pentobarbital and clamping of the aorta was repeated 3 to 4 times with 10 minutes intervals. Table 1 shows that the maximum deflection caused by asphyxiation of the cord is depressed more

TABLE 1

NO.	PRENARCOTIC ASPHYXIATIONS		50 MGM./KGM.	100 MGM./KGM.	150 MGM./KGM.	200 MGM./KGM.
	A	B	C	D	E	F
1	11.4	11.0	8.4 75%	7.3 65%	6.2 55%	4.2 38%
2	3.7	3.3	2.3 66%	1.3 37%	1.1 32%	1.0 29%
3	4.0	4.3	2.3 56%	1.7 41%	0.9 22%	—
4	6.7	7.7	6.2 86%	5.0 69%	2.0 28%	1.7 24%
5	3.6	3.6	2.6 72%	2.1 58%	1.5 42%	1.0 28%

Progressive decrease of the asphyxial depolarisation potential caused by the administration of increasing amounts of pentobarbital. The potentials are given in millivolts.

and more by each subsequent injection. In the 5 experiments of table 1, the average depression of the asphyxial deflection caused by the first narcotic dose of pentobarbital was about 30 per cent.

It has been argued before (van Harreveld, 1946) that the galvanometer deflection caused by asphyxiation of the cord is in general a complex phenomenon, and that the maximum of the deflection reached after 1.5 to 2.5 minutes may be an indication not of the total depolarisation of the most sensitive parts of the neuron (nerve cells), but of the beginning of the depolarisation of more resistant parts (fibers). This will tend to decrease the galvanometer deflection, since complete depolarisation of cells as well as fibers will theoretically cause equipotentiality between the gray matter and the anterior root. The maximum of the depolarisation potential thus is not too reliable a measure for the polarisation state of nerve cells, since this value will be influenced by variations in the start of the fiber depolarisation. Evidence for this has been found in experiments

in which the aorta was clamped at 10 minute intervals without administration of drugs. In one such experiment 5 clampings of the aorta gave the following maxima: 5.1, 5.3, 4.6, 4.4 and 6.7 mV. The maxima of the 3rd and 4th clampings were reached quicker than those of the 1st and 2nd clampings, but the maximum of the 5th clamping was reached considerably later than that of the first. The variations observed in this series are probably not related to changes in the polarisation state of the spinal cord, but to variations in the time at which depolarisation of the various neuron parts begins and in the speed with which this progresses. These considerations have made it necessary to discard a number of experiments in which after the injection of pentobarbital the maximum deflection not only became smaller, but also was reached earlier. In these cases it might be that an earlier depolarisation of the fibers had at least contributed to the decrease of the maximum. In the 5 experiments collected in table 1 the maximum was reached at either the same or a later time during the 6th than during the 1st clamping of the aorta. The decrease of the maximum in these experiments thus indicates that pentobarbital causes an actual decrease of the polarisation state of the most sensitive part of the neuron (cells).

Since it has been concluded that a change in the depolarisation state caused by administration of pentobarbital has to be uniform in the entire neuron, the demonstration of a depolarisation of the cells makes it necessary to assume that the fibers in the cord also depolarise under the influence of this drug. It has been possible to obtain evidence for this in the following way. A silver-chloride-plated silver electrode shaped like a small knife was stuck into the dorsal column of the cord. Another electrode was placed on the undamaged posterior column about 1 cm. distant from the first. An injury potential was led off with these electrodes, the damaged place being negative. After placing the electrodes, a considerable drift of the galvanometer was recorded, indicating a decay of this potential. In some of the experiments, however, the drift decreased sufficiently to allow examination of the effect of pentobarbital on the injury potential. The injury potential is an indication of the polarisation state of the undamaged parts of the nerve or fiber tract, and thus any agent which depolarises these parts will decrease this potential. In a number of experiments it has indeed been found that the injection of pentobarbital causes a definite and permanent decrease of the injury potential. The most convincing record has been redrawn on a convenient time scale in figure 1. A large dose of pentobarbital (90 mgm./kgm. body weight) was injected three times. The first two injections caused considerable decrease of the injury potential, while the third injection had only a small effect. This effect on the injury potential supports the conclusion that pentobarbital depolarises the neuron uniformly.

The effect of ether on the polarisation state of the spinal cord. Ether was administered to preparations in which the polarisation state of the spinal cord had previously been examined by clamping the aorta. The ether usually did not cause any galvanometer deflection; in some experiments small deflections were recorded in either direction. The lack of a consistent effect of ether administration is observed only in experiments in which it is possible to prevent a large

drop of the blood pressure by the infusion of adrenaline. When the aorta was clamped during or immediately after etherization, the maximum of the depolari-

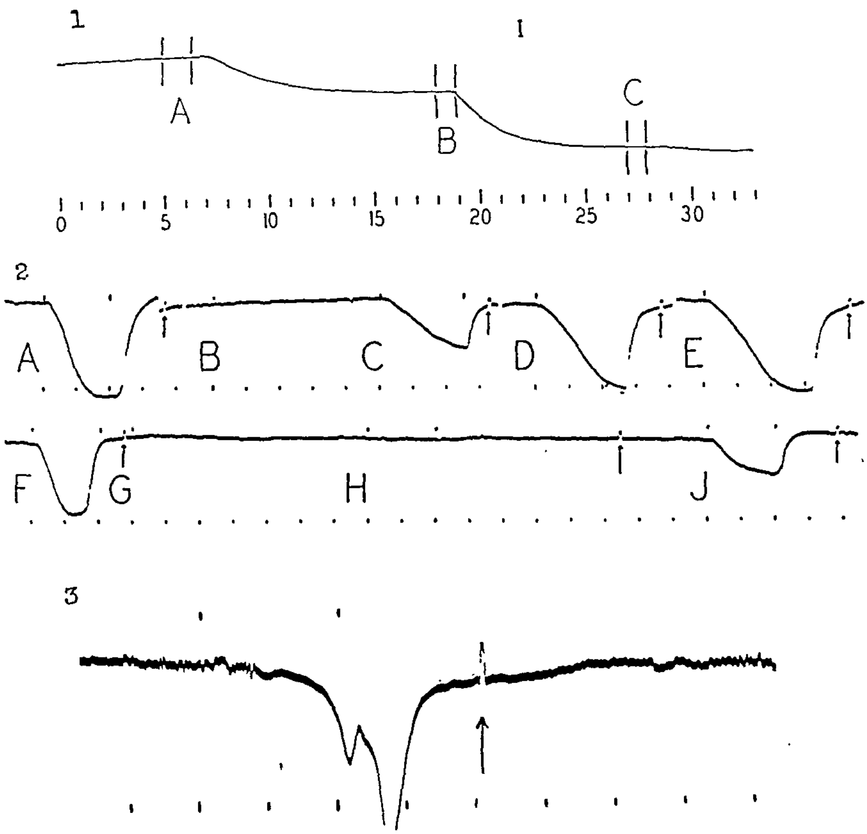

Fig. 1. Effect of pentobarbital on the injury potential of the dorsal column. At *A* 90 mgm. of pentobarbital/kgm. body weight is injected. At *B* and *C* this is repeated. Time in minutes. The vertical line indicates the deflection of 1 millivolt.

Fig. 2. Upper record: effect of relatively light ether narcosis on the asphyxial depolarisation potential. *A*, prenarcotic depolarisation potential. Ether is administered from *B* to *C*. At *C* asphyxiation, immediately following etherization. Between *C* and *D* there is a 10 minute interval during which ether is eliminated. Between *D* and *E* also a 10 minute interval. Minutes are indicated at the bottom of the record; beginning and end of asphyxiation and of administration of ether at the top. The arrows point to 1 millivolt calibrations.

Lower record: Effect of deep etherization on the asphyxial depolarisation potential. This record is continuous. *F*, prenarcotic depolarisation potential. Between *G* and *H* the animal is etherized maximally. Two minutes' asphyxiation is started at *H*. Between *H* and *J* no ether is given. At *J* another 2 minutes' asphyxiation. Signals the same as upper record.

Fig. 3. Cortical asphyxial depolarisation potential. Minutes are indicated at the bottom, beginning and end of a 2 minutes' respiratory arrest at the top of the record. The arrow indicates a 1 millivolt calibration.

sation potential was always decreased markedly as compared with the prenarcotic deflection. Figure 2 shows the records of two experiments; in one (A, B, C, D and E) narcosis was relatively light, in the other (F, G, H and J) it was

very deep. The administration of ether did not produce a significant galvanometer deflection in either of these experiments. The light ether narcosis caused a decrease of the asphyxial depolarisation potential recorded immediately after 5 minutes' ether administration. The depolarisation potential recorded 10 minutes after stopping etherization had grown again to almost the prenarcotic size. The changes in the polarisation state observed during a relatively light ether narcosis are thus easily reversible. In the second record of figure 2, clamping of the aorta after 7 minutes' maximal etherization had practically no effect. When the clamping was repeated 10 minutes after the end of etherization, a considerable depolarisation potential was again recorded. After such deep narcosis the depression of the depolarisation potential is in general not completely reversible for the 30 to 40 minutes following etherization. In a number of experiments the observation was made that although the depolarisation potentials recorded 10 minutes after the end of a deep etherization showed a fair increase, the subsequent clampings of the aorta at 10 minute intervals caused smaller and smaller galvanometer deflections. It is of interest to note that Wright (1947) observed irreversible effects of concentrated ether vapor on the polarisation state of peripheral nerve. Jowett and Quastel (1937), who found that ether inhibits brain respiration in vitro, remarked that this inhibition was reversible only when the exposure of the brain tissue to ether had been slight. The not readily reversible depolarisation of the cord was caused by the application for 7 to 8 minutes of ether in the highest concentration compatible with an undisturbed heart action. Although it was impossible to check reflexes or respiration in the curarised animal, it seems likely that the narcosis produced in this way is deeper than the deepest surgical narcosis.

Often the maximal asphyxial deflection is reached later after etherization than before (see fig. 2). It is possible that this is related to the depression of the metabolism observed by Jowett and Quastel (1937). On the other hand, as mentioned above, similar changes have been observed in series of aorta clampings without the administration of drugs.

Since the administration of ether, like that of pentobarbital, does not produce a consistent potential difference between the gray and white matter of the cord, even though it depresses the asphyxial depolarisation potential, it can be concluded that this narcotic likewise causes a uniform depolarisation of the entire neuron.

Depolarisation of the cerebral cortex by asphyxia and by anesthetics. In some experiments an attempt was made to record the asphyxial changes in the polarisation state of the cerebral cortex. One silver-chloride-plated silver electrode was placed in the gray matter of the cortex, while another was placed on the cortex or in the subcortical white matter. The cortex was asphyxiated either by stopping the artificial respiration or by clamping the innominate and right subclavian arteries in the chest. These procedures caused galvanometer deflections which, although undoubtedly due to the depolarisation of nervous elements in the cortex, lacked the simplicity and constancy of the depolarisation potentials of the spinal cord. Not even the direction of the potential was constant. It is likely that the variations of the cortical depolarisation potentials

are mainly due to differences in placement of the electrodes, especially of the electrode in the cortical gray matter. In four suitable experiments the latent period of the depolarisation potential after stopping the artificial respiration was around 50 seconds. The same value was found previously with this method of asphyxiation for the depolarisation potential of the spinal cord (van Harreveld, 1946). The latent period was considerably shorter when the brain was asphyxiated by clamping the large thoracic arteries.

Figure 3 shows a record of a cortical depolarisation potential. A frequent observation was the uneven baseline, which has not been noticed in similar records of the spinal cord. These irregularities probably represent the electroencephalogram. This assumption is supported by the observation that shortly after the beginning of asphyxiation the irregularities disappear, but develop again a few minutes after 2 minutes' respiratory arrest (Sugar and Gerard, 1938). This figure also illustrates the more complicated shape of the asphyxial potentials of the cortex, and their complete reversibility.

Though the cortical depolarisation potential is probably an even less reliable indicator of the state of polarisation then the more constant and simple potentials observed in the spinal cord, the effect of pentobarbital was investigated. This drug was again injected in doses of 50 mgm./kgm. body weight, and after each injection the depolarisation potential was recorded during clamping of the innominate and right subclavian arteries. One such dose decreased the depolarisation potential, but as much as 4 doses of 50 mgm. of pentobarbital per kgm. of body weight were sometimes insufficient to supress the effect of asphyxiation completely. The cortical depolarisation potential therefore seems to be affected by pentobarbital in a similar manner as the depolarisation potential of the spinal cord.

The effect of pentobarbital and ether on kneejerk and flexion reflex. It was of interest to correlate the depolarisation produced by pentobarbital with the effect of this drug on spinal reflexes. Therefore the kneejerk was recorded on one side of a number of spinal animals and the flexion reflex (anterior tibial muscle) on the other side. The stimuli for these reflexes were given alternately at intervals of about 3 seconds. The kneejerk was elicited by an electromagnetic device, which at regular intervals tapped the quadriceps tendon (van Harreveld, 1939); the flexion reflex was caused by short faradic stimulations of the homolateral n. peroneus superficialis. The blood pressure was recorded and was prevented from dropping below 10 cm. of mercury by the infusion of adrenaline.

Pentobarbital was injected in doses of 50 mgm./kgm. body weight into the carotid artery. In all animals examined one such dose extinguished the flexion reflex completely. Half this amount reduced but did not abolish it. A dose of 50 mgm./kgm. body weight in most cases decreased the kneejerk deflections only slightly (fig. 4). To abolish this reflex, considerably more pentobarbital had to be administered. This can be done only when artificial respiration is applied. One hundred to 125 mgm. pentobarbital per kgm. body weight is usually needed to stop the kneejerk completely. This difference in sensitivity of kneejerk and flexion reflex has been found consistently in all animals examined.

Similar experiments were performed with ether. Like pentobarbital, ether

abolishes the flexion reflex earlier than the kneejerk. When after extinguishing the kneejerk the administration of ether was stopped, this reflex recovered first, followed after a considerable interval by the flexion reflex.

DISCUSSION. It has been concluded previously (van Harreveld, 1946) that asphyxiation first depolarises the most sensitive neuron parts, the cells, followed by depolarisation of the more resistent parts, the fibers. In the present paper the conclusion has been drawn that the narcotics, ether and pentobarbital, also cause depolarisation, but that these drugs affect cells and fibers uniformly. There is some evidence which indicates that depolarisation in these two instances is produced in different ways. Spiegel and Spiegel-Adolf (1936-1938) showed by impedance measurements of the brain with alternating currents of different frequencies, that asphyxia causes an increase of membrane permeability to ions, whereas the administration of narcotics causes a decrease of this value. It is obvious that a sufficient increase of the membrane perme-

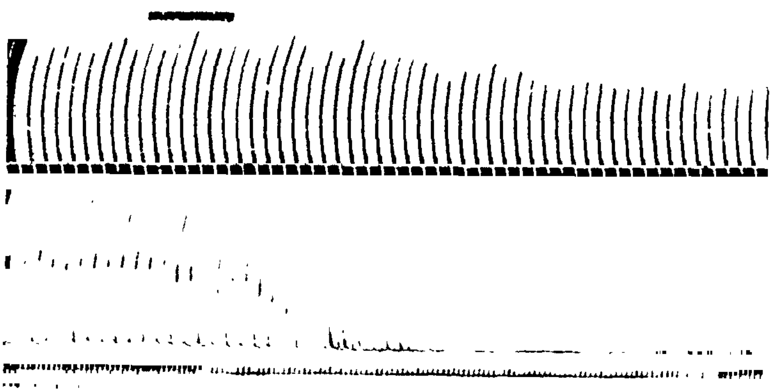

Fig. 4. Effect of the administration of 50 mgm. of pentobarbital per kgm. body weight on the kneejerk (upper record) and on the flexion reflex (lower record). Time in seconds indicated at the bottom of the record. The bar at the top of the record indicates the duration of the injection.

ability may very easily result in a loss of the selective permeability for ions which, according to the membrane theory, is necessary for the maintenance of an electric double layer. A moderate decrease in permeability may, but need not, result in a loss of the selective ionic permeability; it is even conceivable that by becoming less permeable for certain ions than for others the membrane is improved with respect to its ability to produce an electric double layer. The depolarisation of peripheral nerve under the influence of narcotics and local anesthetics observed by Höber, Andersh, Höber and Nebel (1939) and Wright (1947) and the "positive resting potential" resulting from the administration of amyl alcohol and cocaine, found by Bishop (1932) are therefore not necessarily contradictory. The concept that these phenomena are related is supported by Bennet and Chinburg's finding (1946), that both may be observed under the influence of local anesthetics.

It has been observed by several authors (Heinbecker, 1929; Lehman, 1937; Wright, 1946) that asphyxiation of peripheral nerve often causes an initial drop of the stimulus threshold, whereas Wright (1947) found that alcohol and ether

consistently cause a marked rise of this value. The changes in the stimulus threshold may be related to, or even caused by, the increased membrane permeability during asphyxiation, and the decreased permeability during narcosis.

It does not seem likely that the depolarisation produced by narcotics is caused by the depression of the metabolism of central nervous tissue by these drugs (Quastel and Wheatley, 1932, 1934; Jowett and Quastel, 1937; Jowett, 1938), because the depression of the metabolism caused by asphyxia increases the membrane permeability, whereas the narcotics influence the membrane in the opposite direction. It seems more likely that the narcotics act directly on the conducting membrane.

The electrical theory of conduction and transmission (Eccles, 1946) maintains that conduction in the nerve as well as transmission across the synapse is due to the production of a potential difference (action potential) at one place, which stimulates adjacent structures. It seems that ether and probably nembutal influence both these processes in an unfavorable manner for conduction and transmission. Wright (1947) showed in crustacean single fiber preparations that the action potential of depolarising fibers is reduced before blocking occurs. It therefore can be assumed that narcotics reduce the action potentials of nerve fibers and boutons terminaux, which are the stimuli necessary for propagation and transmission. On the other hand, the threshold of stimulation of the conducting membrane is increased by ether (Wright, 1947). These two factors acting together will at a certain point stop the conduction in the nerve or the transmission across the synapse. It is likely that this point is reached earlier at the synapse than in the nerve fiber, since transmission at the synapse is normally less certain than conduction in the fiber. Wright (1947) observed that blocking occurred in peripheral nerve when depolarisation was about 50 per cent complete. In view of the above considerations, pentobarbital in narcotic doses, which reduces the asphyxial depolarisation potential about 30 per cent, could very well block a considerable part of the synapses and thus depress central nervous activity.

It is unlikely that the nervous elements in other parts of the central nervous system than the spinal cord are depolarised by markedly lower concentration of narcotics, since it was found that the effect of pentobarbital on the cortical and on the spinal depolarisation potential is not greatly different. Therefore the difference in sensitivity of reflexes to narcotics is probably not due to differences in the degree of depolarisation of the reflex arc caused by a given concentration of the narcotic, but by differences in the concentration at which the reduced action potential of the boutons terminaux becomes unable to stimulate the less excitable dendrites or nerve cells. Another consideration which is probably of importance for the difference in sensitivity of various reflexes to narcotics is the length and complexity of the reflex arc. The chances that the impulse is arrested at one of the synapses of a multisynaptic reflex arc are greater than in the single synapse of a monosynaptic arc (van Harreveld, 1944). This may be an important factor in the great difference in sensitivity between the multisynaptic flexion reflex and the monosynaptic kneejerk.

To depress the kneejerk completely it was necessary to administer 100 to 125 mgm. of pentobarbital per kgm. body weight. From the 5 experiments of

table 1 it can be computed that this causes an average reduction of the asphyxial depolarisation potential of 45 to 55 per cent. This would indicate a degree of depolarisation of the nervous elements sufficient to explain the suppression of all central nervous conduction.

SUMMARY

1. The administration of the narcotics, pentobarbital, and ether does not produce a consistent potential difference between the gray and white matter of the spinal cord.

2. During asphyxiation of the cord, the gray matter becomes negative with respect to an anterior root. This potential is believed to be due to the depolarisation of nervous elements. During the first minutes of cord asphyxiation it would indicate the depolarisation of the most sensitive neuron parts, the nerve cells. The administration of pentobarbital and ether depresses this asphyxial depolarisation potential.

3. From the depression of the asphyxial depolarisation potential and the absence of an electrical effect of the administration of ether and pentobarbital, the conclusion was drawn that these narcotics cause a uniform depolarisation of the neuron.

4. Asphyxial depolarisation potentials were led off from the cerebral cortex, and were similarly influenced by pentobarbital as the spinal asphyxial potentials.

5. From the effect on the asphyxial depolarisation potential it can be concluded that pentobarbital in narcotic doses causes a sizable depolarisation of central nervous elements.

6. An attempt is made to combine these and other known effects of narcotics on nervous structures in a theory of narcosis.

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EFFECTS OF VITAMIN IMBALANCE UNDER CONDITIONS OF AD LIBITUM FEEDING AND REDUCED CALORIC INTAKE[1]

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In recent years a number of papers have appeared concerning the effects of vitamin imbalance in experimental animals and man. Scandinavian workers (1–3) found that human subjects suffering from multiple dietary deficiencies developed symptoms of niacin deficiency when dosed with thiamine alone. Sydenstricker (4) demonstrated that when niacin alone was given to pellagrins certain signs of their disease, presumably due to a deficiency of other factors, were intensified. Morgan (5) observed that administration of niacin or pantothenic acid alone to dogs receiving "ample amounts of all necessary vitamins except those of the filtrate factors" resulted in a gradual loss of neuromuscular control and sometimes sudden death. All these experiments have one point in common: namely, that the administration of a vitamin resulted in the intensification of symptoms or the precipitation of pathologies that did not occur in animals not so treated. Other investigators, however, were unable to demonstrate adverse reactions following unbalanced dosing even with massive doses of the B-vitamins (6, 7). In view of Richards' suggestion that adverse effects of vitamin imbalance might only be demonstrated under conditions of stress as distinct from growth or maintenance (8), the present study was undertaken to determine the effects of unbalanced dosing both in animals fed (1) *ad libitum* and (2) under the stress of reduced caloric intake.

Procedure and results. Ninety-six female rats of the University of Southern California strain were raised to maturity on a stock ration and selected for the experiment at approximately 90 days of age and an average body weight of 170 grams (range 149 to 196 grams). Two basal rations were employed: diets *A* and *B*. Diet *A* was a purified ration containing the B-complex factors in synthetic form; diet *B* was similar in composition but contained yeast in place of the synthetic B-factors. The synthetic vitamins were incorporated in diet *A* in amounts corresponding to their content in yeast so that the thiamine, riboflavin, pyridoxine, niacin and pantothenic acid content of the two diets was virtually identical. Both diets *A* and *B* were supplemented with massive doses of thiamine hydrochloride, riboflavin and nicotinic acid, the three vitamins employed in the flour enrichment program; and the effects of feeding each of these diets with and without supplements were determined (table 1). Diets were prepared weekly and kept under refrigeration. Animals were fed the above

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rations (1) *ad libitum* and (2) in amounts of 6 grams per day representing a caloric restriction of approximately 50 per cent. Diets were administered daily except Sunday with portions doubled for the Saturday feeding. Animals were weighed weekly, and feeding was continued for 20 weeks (12 animals per group).

Table 1

*Composition of experimental diets**

COMPONENT	DIETS A_1 AND A_2	DIETS B_1 AND B_2
	%	%
Yeast**	0.0	12.0
Vitamin test casein‡	30.0	30.0
Salt mixture‡‡	4.5	4.5
Sucrose	55.5	43.5
Cottonseed oil	10.0	10.0
Vitamin supplements added to diets		
	mgm. %	*mgm.* %
Thiamine hydrochloride	7.2	0.0
Riboflavin	0.9	0.0
Pyridoxine hydrochloride	1.5	0.0
Calcium pantothenate	6.72	0.0
Nicotinic acid	6.0	0.0
2-methyl-napthaquinone	0.5	0.5
Choline chloride	120.0	120.0

Additional supplements of thiamine hydrochloride, riboflavin and nicotinic acid were added to diets A_2 and B_2 in the following amounts per kgm. of diet: thiamine hydrochloride 3.6 grams, riboflavin 0.45 gram and nicotinic acid 3.0 grams.

* All rats were fed once weekly 3 mgm. of alpha-tocopherol and a vitamin A-D concentrate (Nopco Fish Oil Concentrate assaying 800,000 U.S.P. u of vitamin A and 80,000 U.S.P. u of vitamin D per gram) containing 100 U.S.P. u of vitamin A and 10 U.S.P. u of vitamin D.

** Brewers' type yeast #200, Anheuser-Busch, Inc., St. Louis, Mo. Each gram, according to the manufacturer, contained the following vitamin potencies: thiamine, 600 μg; riboflavin 75 μg, pyridoxine 100–125 μg, pantothenic acid 420–560 μg and nicotinic acid 350–500 μg.

‡ Vitamin Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

‡‡ Salt Mixture no. 1, (9).

Results are summarized in table 2. Data were computed on the basis of the top 10 animals in each group with the smallest two in each series eliminated in order to minimize variations in group averages due to infection or atypical responses on the part of individual rats. At least 10 animals in each group survived the experimental period of 20 weeks. When feeding was restricted to 6 grams per day, no significant difference in body weight or gross appearance was observed either on the synthetic or yeast-containing rations between animals fed control diets and those on rations containing massive doses of thiamine hydrochloride, riboflavin and nicotinic acid. For the first 6 weeks of feeding

animals lost weight rapidly on all diets; during the next 6 weeks they continued to lose weight but at a smaller rate. During the last 8 weeks, however, animals regained in virtually all cases part of the weight they had initially lost. After 20 weeks of feeding no significant difference in body weight was observed on any of the four diets employed. On *ad libitum* feeding body weight was greater on control diets than on similar rations containing the thiamine, riboflavin and nicotinic acid supplements. Statistically these differences were not marked but a similar trend was observed on both synthetic and yeast-containing rations. Similarly gain in body weight was greater on diets B_1 and B_2 than on comparable synthetic rations.

TABLE 2

Effects of massive doses of thiamine hydrochloride, riboflavin and nicotinic acid on the body weight of rats fed ad libitum and under conditions of reduced caloric intake

DIETARY GROUP	NO. OF ANIMALS	INITIAL BODY WT. grams	AVERAGE CHANGE IN BODY WT. IN GRAMS AFTER		
			6 weeks	12 weeks	20 weeks
		6 gram per day series			
A_1	10	172.6	−38.9	−48.0	−39.3±4.1*
A_2	10	168.0	−33.0	−44.6	−40.7±3.0
B_1	10	170.5	−39.2	−50.2	−43.2±3.4
B_2	10	168.0	−45.5	−59.0	−51.0±3.7
		Ad libitum series			
A_1	10	168.4	+26.9	+28.1	+42.1±7.2
A_2	10	169.6	+16.2	+21.9	+23.4±4.1
B_1	10	170.5	+37.0	+49.0	+60.6±5.4
B_2	10	171.1	+19.8	+28.9	+41.9±5.2

* Standard error of the mean, $\frac{\sigma}{\sqrt{n}}$.

Effects of massive doses of thiamine hydrochloride, riboflavin and nicotinic acid on the B. M. R. of rats fed ad libitum and under conditions of reduced caloric intake. Inasmuch as thiamine, riboflavin and nicotinic acid are constituents of enzyme systems concerned in cellular oxidation and reduction, the question arose as to what effect massive doses of these vitamins might have on the basal metabolic rate of animals fed *ad libitum* or under conditions of reduced caloric intake. Accordingly, the basal metabolism was determined after 12 weeks of feeding in rats described in the previous section. The apparatus used was a closed circuit type with a capacity of 2 liters (10). Carbon dioxide was absorbed with sodium hydroxide, and oxygen consumption was determined from pressure changes recorded by means of a water manometer. The respiration chambers were kept at 28°C; readings obtained were corrected to standard temperature and pressure. Food was removed from the animals' cages the evening prior

to the metabolism test. At least six successive 5-minute intervals were recorded for each animal, with care being taken to record oxygen consumption when animal activity was at a minimum.[2]

Findings are summarized in table 3. When feeding was restricted to 6 grams per day, no significant difference in basal metabolic rate was observed either on the synthetic or yeast-containing rations between animals fed control diets and those on rations containing massive doses of thiamine hydrochloride, riboflavin and nicotinic acid. In all groups fed a reduced caloric intake oxygen consumption per 100 grams body weight was significantly less than for animals fed similar rations *ad libitum*. On *ad libitum* feeding, however, basal metabolic rates were greater on the control diets than for rations containing the thiamine, riboflavin and nicotinic acid supplements. Statistically these differences were not marked but they occurred on both the synthetic and yeast-containing rations.

TABLE 3

Effects of massive doses of thiamine hydrochloride, riboflavin and nicotinic acid on the B.M.R. of rats fed ad libitum and under conditions of reduced caloric intake

DIETARY GROUP	NO. OF ANIMALS	O_2 CONSUMPTION cc/hr/100 grams BODY WT.	DIETARY GROUP	NO. OF ANIMALS	O_2 CONSUMPTION cc/hr/100 grams BODY WT.
6 grams per day series			*Ad libitum series*		
A_1	8	91.5±9*	A_1	4	131.4±16*
A_2	8	85.2±4	A_2	4	116.0±6
B_1	8	89.8±11	B_1	4	138.0±19
B_2	8	94.4±11	B_2	4	112.2±10

* Average deviation.

Effects of massive doses of thiamine hydrochloride, riboflavin and nicotinic acid. on the blood counts of rats fed ad lib. and under conditions of reduced caloric intake. The suggestion has been made that unbalanced vitamin therapy if sufficiently prolonged may increase requirements for other nutrients to the extent that deficiencies occur. Since leucopenia, granulocytopenia and other abnormalities in the formed elements of the blood may occur in deficiency states, the question arose as to what effect massive doses of thiamine, riboflavin and nicotinic acid might have on the blood count of rats fed *ad libitum* or under conditions of reduced caloric intake. Accordingly animals fed the diets previously described were selected after 12 weeks of feeding, and total and differential white cell counts, hemoglobin determinations and total red cell counts were made on the tail blood of all rats. Differential counts were made on smears stained with Wright's stain, 100 cells on each of two slides being employed for each analysis. All blood counts were made in duplicate.

[2] We are indebted to Dr. R. J. Winzler of the Department of Biochemistry and Nutrition of the University of Southern California for his assistance with the B.M.R. determinations. Readings were made by G.D.M.

No significant difference in total erythrocytes or hemoglobin levels was observed either on synthetic or yeast-containing rations between animals fed control diets and those on rations containing massive doses of thiamine hydrochloride, riboflavin and nicotinic acid. Erythrocytes averaged 7.4 to 8.1 million per cc. of blood for the various groups (range 6.7–9.6 million per cc.), with hemoglobin averaging 15.3 to 15.9 mgm./100 cc. (range 14.0–17.8 mgm./100 cc.). Data on leucocyte counts are summarized in table 4. In agreement with earlier findings (11) a significant reduction in total leucocytes was observed in animals fed reduced caloric intakes. This occurred on both synthetic and yeast-containing rations. The two diets differed, however, in respect to percentage and total granulocytes per cubic centimeter of blood. When feeding was restricted

TABLE 4

Effects of massive doses of thiamine hydrochloride, riboflavin and nicotinic acid on the leucocyte count of rats fed ad libitum and under conditions of reduced caloric intake

DIETARY GROUP	NO. OF ANIMALS	TOTAL LEUCOCYTE COUNT		GRANULOCYTES	
		Average*	Range	Per cent*	Total*
		6 gram per day series			
A_1	10	8,400±450	6,200–10,700	13.8±0.4	1159± 30
A_2	10	8,290±420	7,000–10,800	14.5±1.0	1202± 85
B_1	10	7,440±370	6,300–10,800	25.9±1.0	1927± 74
B_2	10	7,490±620	5,000–11,000	30.4±0.7	2277± 52
		Ad lib series			
A_1	10	13,500±580	11,400–17,200	15.2±1.4	2052±189
A_2	10	13,100±660	9,000–18,800	22.3±2.4	2921±314
B_1	10	13,470±440	11,200–15,600	15.1±0.8	2034±108
B_2	10	14,570±810	10,800–17,400	22.7±0.6	3307± 87

* Including standard error of the mean, $\frac{\sigma}{\sqrt{n}}$.

to 6 grams per day, the percentage of granulocytes on diet B_1 was increased so that total granulocytes per cc. of blood were equal to values observed on this same ration when fed *ad libitum*. No such increase in percentage of granulocytes occurred, however, on diet A_1 when fed under similar conditions of caloric restriction with the result that total granulocytes per cc. of blood were reduced in this series by approximately 50 per cent. No significant difference was observed at this level of feeding either on synthetic or yeast-containing rations between the granulocyte and total leucocyte count of rats fed control diets and animals fed similar rations containing massive doses of thiamine hydrochloride, riboflavin and nicotinic acid. On *ad libitum* feeding, however, percentage and total granulocytes were greater on diets containing the thiamine, riboflavin and nicotinic acid supplements than they were on control rations. Statistically these dif-

ferences were not marked but they occurred on both the synthetic and yeast-containing rations, and at least for the latter appear to be significant. Total leucocytes per cubic centimeter of blood were similar for all diets employed.

DISCUSSION. Available data indicate the nutritional requirements of an animal may be affected by such factors as physical exertion, fever, drugs, toxins, abnormal environmental conditions, pregnancy, lactation, hyperthyroidism and related "stress factors" (12); and that caloric restriction may also serve as such a factor (11). In view of Richards' suggestion that adverse effects of vitamin imbalance may only develop under conditions of stress as distinct from growth or maintenance (8), it was felt that animals fed reduced caloric intakes might demonstrate adverse effects of unbalanced dosing more readily than animals fed similar rations ad libitum. Reduced caloric intake was employed as the stress factor in the present experiment since low calorie diets are widely consumed today by persons unable to obtain sufficient food to meet body requirements as well as by persons on therapeutic regimes; and it was believed desirable to determine the effects of vitamin imbalance under these conditions.

When feeding was restricted to 6 grams per day, no adverse effects were observed on either of the basal rations employed attributable to the administration of massive doses of thiamine hydrochloride, riboflavin and nicotinic acid. Gross appearance, loss in body weight, basal metabolic rate, R. B. C., Hb., total W. B. C. and differential counts did not differ significantly between animals fed control diets and those fed similar rations containing the vitamin supplements. Under conditions of ad libitum feeding no significant difference in gross appearance, R. B. C., Hb., and total W. B. C. count was observed between animals fed the control and vitamin-supplemented diets. The latter group, however, gained less weight, had a lower basal metabolism and a higher granulocyte count per cubic centimeter of blood than animals fed the control rations. These differences although consistent for both the synthetic and yeast-containing diets were not sufficiently marked in most cases to be statistically significant. Findings indicate, at least for animals fed reduced caloric intakes, that under conditions of the present experiment vitamin imbalance, as caused by massive doses of thiamine, riboflavin and nicotinic acid, did not result in adverse effects in the female rat.

SUMMARY

Female rats were maintained for 20 weeks on purified rations and similar diets containing massive supplements of thiamine hydrochloride, riboflavin and nicotinic acid. Two basal rations were employed: one containing the B vitamins in synthetic form and the other providing them as present in yeast. Animals were fed ad libitum and at a caloric restriction of 50 per cent.

Under conditions of ad libitum feeding no significant difference in gross appearance, R. B. C., Hb., and total W. B. C. count was observed between animals fed control diets and those fed similar rations containing the thiamine, riboflavin and nicotinic acid supplements. The latter group, however, gained less weight, had a lower basal metabolism and a higher granulocyte count per cubic centimeter of blood than animals fed control rations.

At a caloric restriction of 50 per cent no significant difference in gross appearance, body weight, basal metabolic rate, R. B. C., Hb., total W. B. C. and differential counts were observed between animals fed control diets and those fed similar rations containing the vitamin supplements.

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ELECTROMYOGRAPHIC STUDIES ON CATS AFTER SECTION A SUTURE OF THE SCIATIC NERVE

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Weddell, Feinstein and Pattle (1) (1943), using concentric needle electrodes (Adrian and Bronk), investigated the electrical activity present in mammalian skeletal muscle (including the human being) after denervation by complete crushing of the nerve supply and during the course of nerve regeneration until functional recovery had taken place. From this and later studies (2, 3), the following facts relative to reinnervation of muscle were evident: 1. There was a steady decrease in the number of fibrillation action potentials prior to the return of motor unit activity, but a few fibrillation action potentials could be recorded in certain positions in the muscle when no detectable weakness could be recorded clinically. 2. There was a cessation of continuous fibrillation immediately before the appearance of nascent (polyphasic) motor unit action potentials. 3. Once started, the spread of motor unit activity throughout the muscle occurred quite rapidly, requiring only 5 to 6 days in the rabbit, and 12 days in the human being. 4. When no electrical activity whatever could be recorded from a muscle more than a few weeks after nerve injury (complete electrical silence), severe morphological changes were assumed to have taken place, e.g., fibrosis. 5. Complete functional recovery did take place in muscles proven to be fibrillating by electromyography for various time intervals after denervation.

Jasper (4), employing the monopolar needle electrode, investigated the time course of electrical activity in patients with spontaneously recovering peripheral nerve lesions and also in patients following nerve suture. In this study, he likewise found the first sign of reinnervation to appear in the electromyograph as a decrease or cessation of denervation fibrillation, but in addition, found complete electrical silence in some muscles several weeks before the appearance of motor unit activity. This latter fact he believed showed that the regenerating nerve may arrest fibrillation in the muscles before it has matured sufficiently to conduct any impulses. To this end, he computed the rates of reinnervation for various nerves using both electromyographic silence and return of motor unit activity in muscles as signs of reinnervation.

While both Weddell and Jasper observed a decrease in the amount of denervation-fibrillation prior to beginning motor unit recovery, Weddell's obser-

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vation concerning the cessation of continuous fibrillation before motor unit recovery appears more critical. This is true because Weddell has emphasized the impossibility of measuring quantitatively the decrease in denervation-fibrillation without an excessive number of needlings. Jasper's observation, relating complete electrical silence with successful neurotization of muscle rather than with severe morphological changes in it, could be of great importance in clinical diagnosis and prognosis of peripheral nerve injuries. Jasper, however, has neither discussed the spread of motor unit activity in muscles following nerve suture nor has he reported complete functional recovery in muscles proven to be fibrillating by electromyography during the period of denervation following nerve suture.

Because of the several discrepancies and deficiencies mentioned above, an experiment was devised which would permit a statistical study of the electrical activity in the cat's tibialis anticus muscle following section and immediate suture of the sciatic nerve until functional recovery was complete.

MATERIAL AND METHODS. The material consisted of 6 cats all of which had, under nembutal anesthesia, a section and an immediate suture of the left sciatic nerve approximately 4 inches (102 mm.) from the motor point of the tibialis anticus muscle. At each examination, 4 areas (proximal to distal) of each muscle received a single insertion of a needle electrode, thus making a total of 24 areas sampled. Using Tippett's random number tables, a program was prepared which ensured the obtaining of data in random sequence. The temperature of the examining room was maintained at about 75°F., and no pharmacological agents were administered to the animals during the course of this investigation.

The first examinations were made on the 8th postoperative day and thereafter at frequent intervals until the 90th postoperative day at which time there was no clinical evidence that complete functional recovery had not occurred. The animals were sacrificed on the 90th postoperative day and subsequent histological studies on the distal segments revealed good axis cylinder and myelin sheath formation in all but animal 4.

The electromyograph used for this investigation was essentially the same as the one described by Jasper and Johnston (5). It consisted of a preamplifier, power amplifier, cathode ray oscilloscope, and loud speaker. Electromyograms were obtained by photographing the tracings on the oscilloscope. Using the monopolar insulated steel needle electrode with reference to an adjacent skin surface lead, we found the action potentials or voltages in cat's normal, denervated, and reinnervated skeletal muscle to have the following characteristics.

Normal motor unit action potentials or voltages. Normal voluntary muscle in a completely relaxed state generates no voltages of sufficient magnitude to be recorded by the electromyograph. Normal voluntary muscle in a state of contraction generates voltages which are usually diphasic in character and range in magnitude from about 500 to 2000 microvolts. The duration of a single diphasic wave is in the order of 5 to 10 msec., and the repetition frequency varies from about 5 to 30 per second. These voltages called "normal motor unit voltages"

are readily obtained from all areas of a normal muscle, and because of the long duration of a single wave, they produce a characteristic thumping noise in the loud speaker (fig. 1-B).

Fibrillation action potentials or voltages. The voltages elicited from denervated voluntary muscle are commonly referred to as "fibrillation voltages" and may be either monophasic or diphasic in character. They range in magnitude from about 10 to 100 microvolts, and their repetition frequency varies from about 2 to 30 per second. A single wave has a duration of approximately 1 to 2

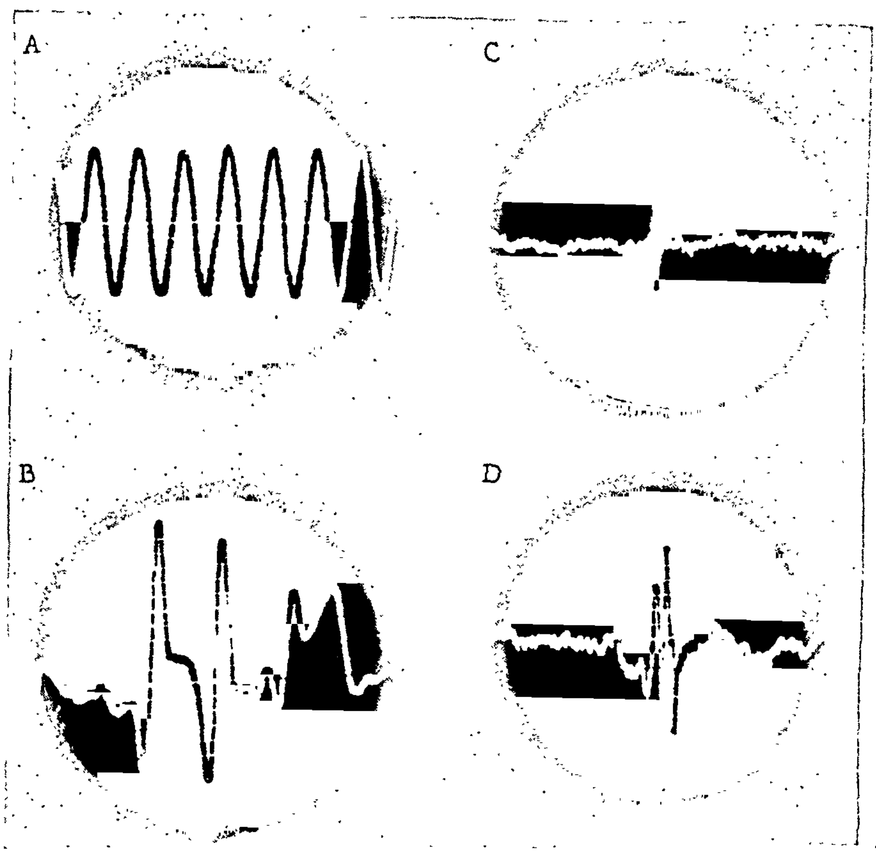

Fig. 1. Oscillograms of typical waveforms

msec., and because of its extremely short duration, such a wave produces a very characteristic clicking noise in the loud speaker (fig. 1-C).

Nascent (polyphasic) motor unit action potentials or voltages. During the period of reinnervation, polyphasic waves are elicited the peaks of which are usually very spiked in character. These polyphasic waves called "nascent motor unit potentials" by Weddell, range in magnitude from about 20 to 600 microvolts. Their repetition frequency varies from about 2 to 30 per second, and the duration of a single wave group is usually in the order of 5 to 15 milliseconds. Because of the complexity of these waves, nascent motor unit voltages give rise to a very rough sounding noise in the loud speaker (fig. 1-D).

EXPERIMENTAL RESULTS. *Onset and course of fibrillation voltages.* In order to illustrate in graphic form the onset and course of fibrillation, a bar chart (fig. 2) has been constructed. In this chart the height of the black bars indicates the number of areas having continuous fibrillation voltages expressed as a percentage of the total number of areas sampled on any designated postoperative day. Similarly, the height of the bars with the vertical lines indicates the percentage of areas having discontinuous fibrillation voltages. Fibrillation voltages were designated as continuous if they persisted for 30 seconds or longer after the insertion of the needle electrode, and discontinuous if they lasted for less than 30 seconds.

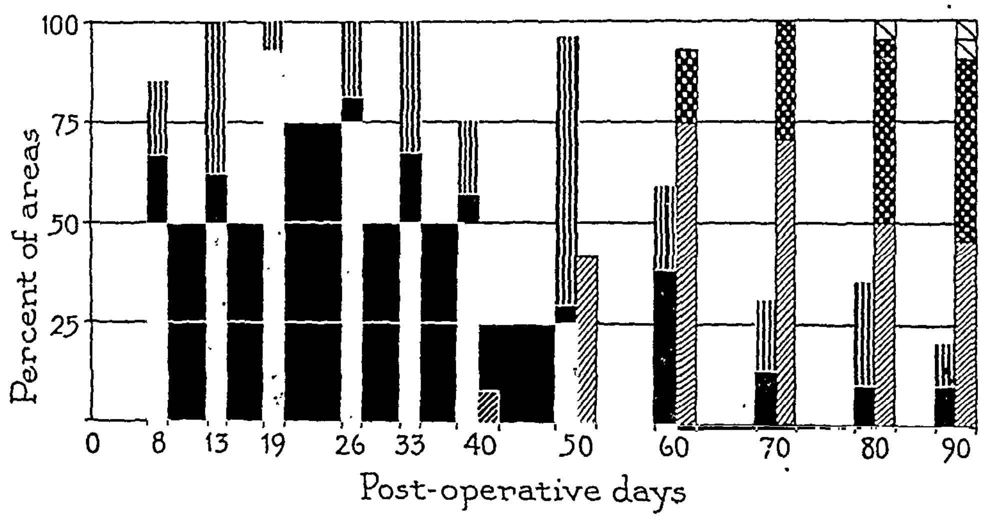

Fig. 2. Bar chart showing number of areas having fibrillation and motor unit voltages expressed as a percentage of the total number of areas examined on designated postoperative days.

Reference to figure 2 shows that on the 8th postoperative day, 65 per cent of the 24 areas had continuous fibrillation and 20 per cent had discontinuous fibrillation, thus making a total of 85 per cent of the areas sampled showing some fibrillation voltages. The remaining 15 per cent of the areas, however, exhibited complete electrical silence on the 8th postoperative day. On the 13th day fibrillation voltages were obtained from 100 per cent of the areas; this condition was also present on the 19th, 26th, and 33rd postoperative days. By the 40th day only 75 per cent of the areas showed fibrillation voltages; however, an examination 10 days later revealed 91.6 per cent of the areas to be fibrillating. Subsequent examinations on the 60th, 70th, 80th, and 90th postoperative days showed a gradual decline in the percentage of the areas having fibrillation voltages. Even on the 90th postoperative day, however, 20 per cent of the areas contained denervated muscle fibers as evidenced by fibrillation voltages.

In this experiment we, like Weddell et al. (2) and Jasper (4), observed a definite decrease in the number and frequency of fibrillation voltages prior to beginning motor unit recovery, but were unable to measure this decrease with any degree of accuracy, and consequently, we are unable to present it in graphic form.

As seen in figure 2, the number of areas having *continuous* fibrillation reached a maximum on the 19th day (91.6 per cent) after which there was a gradual decrease in the number, so that by the 90th day only 10 per cent of the areas showed fibrillation of the continuous type. Although there was a significant decrease in the total number of areas having continuous fibrillation between the 19th and 50th postoperative days, no one muscle showed a cessation of continuous fibrillation in all four areas prior to beginning motor unit recovery.

Electrical silence. Muscles having neither fibrillation nor motor unit voltages are said to be electrically silent. While on the 8th postoperative day electrical silence was observed in 15 per cent of the areas, it was not noted again until the 40th day at which time 16.6 per cent of the areas showed complete electrical silence. On the 50th day, however, fibrillation voltages were again elicited from one of the previously silent areas, fibrillation and motor unit voltages from two of them, and motor unit voltages only from the remaining one. In three, then, motor unit voltages were seen to follow the previous electrical silence, but in three fibrillation voltages reappeared. In this regard attention must be drawn to the fact that at no time was electrical silence found in all four areas of any one muscle.

Onset and course of nascent motor unit voltages. The return of motor unit voltages is illustrated in figure 2 by the cross-hatched and checkered columns. The height of these columns indicates the number of areas having motor unit voltages expressed as a percentage of the total number of areas sampled. The type of motor unit voltages obtained is indicated by the direction of the diagonal lines and the combination of nascent and normal forms is indicated by the checkered columns. A consideration of this chart shows that no motor unit voltages were recorded until the 40th postoperative day. However, on the 40th and 50th postoperative days, "nascent" motor unit voltages were elicited from 8.3 per cent and 41.6 per cent of the areas, respectively. On the 60th postoperative day, 79.3 per cent of the areas had nascent forms, while 12.4 per cent contained both nascent and normal forms. Subsequent examinations on the 70th, 80th, and 90th postoperative days revealed motor unit activity in 100 per cent of the areas on each occasion, and in addition, revealed a decrease in the number of areas having nascent forms only, and an increase in the number of areas having both nascent and normal forms. By the 80th postoperative day, 5 per cent of the areas had normal forms only, and on the 90th postoperative day, normal forms only were elicited from 10 per cent of the areas.

Although 30 days elapsed from the appearance of the first motor unit voltages until they could be elicited in 100 per cent of the areas, the range of time required for the spread of motor unit activity throughout individual muscles (motor unit spread interval) was only 10 to 20 days, the mean being only 11.66 days. This apparent discrepancy is explained on the basis that only one muscle had beginning motor unit recovery as early as the 40th postoperative day, whereas the other five did not have beginning motor unit recovery until the 50th postoperative day. Furthermore, the motor unit spread interval equaled 20 days in one muscle which did not show beginning motor unit recovery until the 50th postoperative day.

The appearance of "nascent" motor unit voltages preceded unequivocal functional recovery by different intervals of time varying from 9 to 16 days. In assessing the return of voluntary motion, a minimal movement of the tibialis anticus tendon was taken as the end-point. In general, when motor unit voltages were recorded on the 50th postoperative day, functional recovery was definite at about the 62nd postoperative day. When functional recovery was definite, five of the muscles had motor unit activity in all four areas, and the remaining muscle had this activity in two areas. Once started, functional recovery progressed very rapidly; consequently, no weakness could be detected by clinical methods in any of the muscles on the 90th postoperative day.

Rate of reinnervation as assessed by electromyography and return of voluntary motion. Although Guttman et al. (6) and Seddon et al. (7) have pointed out the many factors to be considered in establishing the rate of reinnervation of muscle, we have elected to use Jasper's (4) simplified method for the purpose of making comparisons with his data. This consists of dividing the length of the distal segment in millimeters by the time elapsed since surgery in days for beginning motor unit recovery, subtracting 10 days for delay at the scar. Using the above formula and beginning motor unit recovery as the first sign of reinnervation, we have found the rate of reinnervation to range from 2.52 mm. per day to 2.96 mm. per day, the mean being 2.61 mm. per day. Similarly, when a detectable movement of the muscle was used as the first sign of reinnervation and applied to the above formula, the rate of reinnervation was found to range from 1.8 mm. per day to 2.26 mm. per day, the mean being 2.02 mm. per day.

Discussion. In this investigation fibrillation voltages were recorded throughout the lengths of all of the muscles for various intervals of time after denervation, and in particular, continuous fibrillation voltages were recorded for various time intervals. As reported previously by Weddell et al. (2) and Jasper (5), a definite but unmeasurable decrease in the number and frequency of fibrillation voltages was noted prior to beginning motor unit recovery. While we did observe a steady decrease in the number of areas exhibiting continuous fibrillation prior to beginning motor unit recovery, we failed to find a cessation of continuous fibrillation in all areas of any one muscle as reported by Weddell (1).

Although electrical silence was observed in 15 per cent of the areas on the 8th postoperative day, fibrillation voltages were recorded five days later from all of these previously silent areas. Of the four electrically silent areas observed on the 40th postoperative day, motor unit voltages only were elicited from just one on the 50th postoperative day, and at no time was electrical silence found in all four areas of any one muscle. From these data on the experimental animal (cat), electrical silence has not been found to bear a close correlation with either severe morphological changes in muscle or beginning motor unit recovery as reported by Weddell et al. (1) and Jasper (4), respectively. From the evidence at hand, no interpretation should be placed on electrical silence per se unless the presence or absence of muscle tissue is first ascertained by some other means, e.g., electrical studies or muscle biopsy.

The motor unit voltages associated with beginning motor unit recovery were in all instances of polyphasic wave form as reported by other workers. The

time interval required for the spread of motor unit activity throughout the muscles (motor unit spread interval) ranged from 10 to 20 days, the mean being 11.66 days. The duration of the motor unit spread interval after nerve suture in the cat was somewhat longer than the one reported by Feinstein et al. (3) after nerve crushing in the rabbit (5 to 6 days), and about the same as the one reported by these workers after nerve crushing in the human being (12 days). The explanation for these differences in duration of the motor unit spread interval in various mammals is not at hand, but the lengths of the muscles and the species of the mammal are undoubtedly contributing factors. Although complete functional recovery did follow in all muscles having a relatively short motor unit spread interval, more evidence is needed to presage an inverse relationship between the duration of the motor unit spread interval and the percentage of functional recovery.

Beginning motor unit recovery preceded unequivocal functional recovery in all instances, and when unequivocal functional recovery was present, motor unit voltages were elicited from all four areas of five muscles, and from two areas in the sixth. The time interval between beginning motor unit recovery and unequivocal functional recovery (functional delay interval) ranged from 9 to 16 days in the cat with a mean of 12 days. Weddell et al. (2) observed the functional delay interval to range from 2 to 15 days after nerve crushing, and from 90 to 120 days after delayed suture in the human being. They consider the duration of the functional delay interval to be a function of several factors including "quality" of reinnervation, anatomical position and action of the reinnervated muscles, and the change in destination of many axons in the course of reinnervation. However this may be, they do report complete functional recovery in the muscles after nerve crushing, but have failed to state when and if it did occur after delayed suture. In one case of delayed suture in which the functional delay interval equaled 14 days, they did observe good power in the muscle 35 days after the first detectable movement. Again, insufficient evidence is at hand to presage an inverse relationship between the functional delay interval and the percentage of functional recovery; however, complete functional recovery either occurred or was anticipated in all instances when the functional delay interval was of relatively short duration.

Once started, functional recovery progressed very rapidly and by the 90th postoperative day no weakness could be detected clinically in any of these muscles. This fact is of particular interest because fibrillation voltages were recorded from two of the muscles, and polyphasic (nascent) motor unit voltages were recorded from some areas in all of them on the 90th postoperative day. In other words, complete functional recovery preceded complete electromyographic recovery in all instances.

Using beginning motor unit recovery as a sign of reinnervation, we have found the rate of reinnervation to range from 2.52 to 2.96 mm. per day with a mean of 2.61 mm. per day. Jasper (4) found this rate for the peroneal nerve in the human being to range from 0.6 to 3.4 mm. per day with a mean of 1.6 mm. per day. For all other nerves (ulnar, radial, median, and posterior tibial) he found this

rate to range from 0.8 to 5.0 mm. per day with a mean of 2.56 mm. per day. Although Jasper has not reported a direct relationship between the rate of reinnervation and the percentage of functional recovery, he does report an inverse relationship between the rate of reinnervation and the time interval between nerve injury and nerve suture (suture delay interval). Young (8) reported an inverse relationship between a prolonged suture delay interval and the effectiveness of the result, and considers shrinking of the schwann tubes and atrophy of the muscles to be of paramount importance in preventing an effective result. From the work of Young (8) and Jasper (4), one may therefore deduce a direct relationship between the rate of reinnervation and the percentage of functional recovery. Whether or not a slow rate of reinnervation would in all instances presage incomplete functional recovery, we cannot say at this time, but we did observe complete functional recovery in all muscles when the average rate of reinnervation (using beginning motor unit recovery as a sign of reinnervation) was 2.61 mm. per day.

Although Langley (9) (1916) and Tower (10) (1939) considered denervation fibrillation to contribute markedly to denervation atrophy, more recent work (11) and Feinstein et al. (3) failed to show the existence of such a relationship between denervation fibrillation and denervation atrophy. However this may be, too much emphasis has been placed upon the rôle of fibrillation as related to atrophy and too little upon the rôle of fibrillation as related to ultimate functional recovery. In the present experiment, we observed complete functional recovery in all muscles even though they were proven by electromyography to be fibrillating for various time intervals after denervation. Weddell et al. (2) (1944) found continuous fibrillation to be absent in denervated muscles immobilized for prolonged periods of time (5 mo.), but were able to re-establish continuous fibrillation in these muscles with vigorous physiotherapy in the form of radiant heat, massage, galvanic stimulation, and passive movements.

Chor et al. (12) made histopathological studies on denervated muscle (macacus rhesus monkey's gastrocnemius-soleus) immobilized for various periods of time, and reported longitudinal striations to be apparent as a result of cleavage between small bundles of myofibrillae two weeks after denervation. At the end of six weeks the degeneration of the myofibrillae was very prominent and the striations were no longer visible in many fibers. At this time the muscle cells had been converted into granular structures in which vacuoles were seen as evidence of severe breakdown. Twelve weeks after denervation there was marked fibrosis of the degenerated muscle fibers; however, a few residual segments of muscle fibers undergoing transformation into fibrous tissue were noted.

Tower (13) (1935) reported the progressive histological changes in non-immobilized skeletal muscle (cat's interossei) following denervation, but observed a generally normal appearance of the contractile element of the muscle fibers—the myofibrillae—two weeks after denervation. Two months after denervation a fading of the cross striations was noted but the contractile and the orienting mechanism were still in working order. At four months Tower reports the emergence of "fibrotic de-differentiation." By comparing these progressive

histological changes in immobilized and non-immobilized skeletal muscle, one is impressed with the rapid rate of degeneration in the immobilized muscles.

While a species difference may conceivably account for part of the rapid degeneration in these immobilized muscles, immobilization itself appears to have been the fundamental factor. This fact is given additional support by the work of Huddleston et al. (14) who observed prolonged immobilization of denervated muscle (dog's tibialis anticus and gastrocnemius-plantaris) to interfere in some way with subsequent reinnervation and ultimate functional recovery. Although these workers did not study the electrical activity of the muscles, the findings of the present experiment together with those of Weddell et al. (2) entitle one to presume the absence of continuous fibrillation in the immobilized muscles and its presence in the non-immobilized controls.

From the evidence presented one may adduce that continuous fibrillation is a physiological response of skeletal muscle to denervation, and for this reason it is of paramount importance in maintaining denervated skeletal muscle as contractile tissue during the denervation period. Expressed somewhat differently, fibrillating denervated skeletal muscle is contractile tissue having the physical capacity for reinnervation. With these thoughts in mind, there appears to be a sound physiological basis for prescribing only those physical and chemical agents, during the period of denervation, which are known to enhance continuous fibrillation.

SUMMARY

An investigation was made concerning the time course of electrical activity in the tibialis anticus muscles of six cats after section and immediate suture of their nerve supply.

Fibrillation voltages were recorded throughout the lengths of all muscles for various time intervals following denervation. There was a definite but unmeasurable decrease in the number and frequency of fibrillation voltages prior to beginning motor-unit recovery. There was a decrease in the total number of areas showing continuous fibrillation, but there was no cessation of continuous fibrillation in all areas of any one muscle prior to beginning motor-unit recovery.

Electrical silence did not bear a close correlation with either severe morphological changes in muscle or beginning motor-unit recovery. The motor-unit voltages associated with beginning motor-unit recovery were in all instances of polyphasic wave form.

The average duration of the motor-unit-spread interval was 11.66 days. Beginning motor-unit recovery preceded unequivocal functional recovery in all instances. Motor-unit voltages were elicited from all four areas in five muscles and from two areas in the sixth when the muscle showed unequivocal functional recovery. The average duration of the functional delay interval was 12 days.

Complete functional recovery preceded complete electromyographic recovery in all instances. The average rate of reinnervation (using beginning motor-unit recovery as a sign of reinnervation) was 2.61 mm. per day. Complete functional recovery occurred in all muscles even though they were proven by electromyography to be fibrillating for various time intervals after denervation.

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REFRACTIVE CHANGES PRODUCED BY INJECTION OF FLUIDS INTO THE VITREOUS HUMOR

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Osmotic disturbances in body fluids have been shown to give rise to lenticular changes in the eye, such as the cataracts which appear in the rat within a space of minutes after intravenous injection of galactose (1). The transient cataracts in rats after decompression are also thought to be due to an osmotic upset resulting from an increase in the lactic acid content of the aqueous (2). Cataracts also develop gradually in rats maintained on a diet containing a large percentage of lactose or galactose (3). The galactose-fed rats develop cataracts earlier than lactose-fed rats, and the speed at which the cataracts develop depends on the percentage of galactose in the diet (4). It is claimed that in this condition there is a decreased permeability of the lens capsule, and this accentuates the osmotic difference between the lens and aqueous humor (5).

The high incidence of cataract and refractive change in human diabetics has therefore been attributed to abnormal osmotic relations of the body fluids consequent upon the high blood sugar. In the course of an investigation of this question it was desired to ascertain whether *local* osmotic changes within the eyeball as well as general systemic osmotic disturbances could cause lenticular or refractive changes.

Cats under nembutal anesthesia were chosen for the experiment, since with atropine cyclopegia the cat's eye can be readily refracted, and the range of accommodation upon nerve stimulation has been found to be as much as 8.00 D under these conditions. In a few instances dogs under nembutal were also used. For the experiment the skin at the lateral canthus was cut away sufficiently to expose a small area of sclera, and by rotating the globe nasalwards, a portion posterior to the equator was made accessible. Injection of fluids of various tonicities was made into the vitreous. It was found that corneal clouding resulted from the increased intra-ocular pressure when the volume of the injected fluid exceeded 0.25 cc. Therefore volumes of 0.2 cc. were used in all the tests and in no case was corneal clouding observed with injections of this amount of fluid. Both hypo- and hypertonic solutions were used, on the assumption that if any changes did occur and these were the result of altered osmotic relations, they would vary with the tonicity of the injected fluid. The solutions tried were 1, distilled water; 2, physiological saline or Ringer's solution; 3, 1.5 per cent NaCl, and 4, a glucose solution equivalent osmotically to the previous salt solution. Refractive changes were determined by retinoscopy and the ocular media were examined ophthalmoscopically.

Injection of 0.2 cc. of each of the above fluids into the vitreous produced the same effect in every case, viz., a transient refractive change of 1.00 to 3.00 D in the direction of hypermetropia, but there was no indication of even transient

cataract. This induced hypermetropia regressed rapidly in the first few minutes, then more gradually until the normal refractive value was reached in from 12 to 20 minutes after the injection. Typical results are shown in table 1.

It is evident that the tonicity of the injected fluid is not the important factor in bringing about this refractive change. Neither is it the result of trauma, since merely piercing the globe with a needle without injecting any fluid produces no demonstrable refractive change in the eye of a deeply nembutalized cat. The reaction does not involve the sympathetic nerve supply to the eye—stimulation of which causes the lens to flatten and thus to produce hypermetropia (6)—since the result was obtained on injection of fluid into the vitreous after the corresponding superior cervical sympathetic nerve had been severed in the neck. It does not appear to be the mechanical result of increased pressure distorting the shape of the globe, since the effect is in the direction of hypermetropia, not myopia as one would expect from a stretching of the envelope of the eyeball. Nor is it the result of a forward displacement of the lens since this should also produce myopia rather than hypermetropia.

TABLE 1

	SOLUTION INJECTED	REFRACTIVE CHANGE IN DIOPTERS
Cat 1	R. eye, 1.5% NaCl L. eye, normal saline	+1.00 +1.50
Cat 2	R. eye, distilled water L. eye, normal saline	+1.25 +0.75
Cat 3	R. eye, dextrose solution L. eye, normal saline	+1.50 +1.25

There remains the possibility that some vascular change within the eyeball might affect the shape of the lens, such as the forcing of blood out of the relatively rigid globe by the introduction of fluid under pressure into it. If this were the case, there might be some correlation between the degree of hypermetropia induced and the state of the blood vessels within the globe, or the ease with which blood could leave the eye. Since general vasodilatation may be produced by inhalation of amyl nitrite and general vasoconstriction by intravenous injection of adrenaline, the effect on the phenomenon of these two drugs was tried.

Refraction of the eye of the nembutalized cat while the blood pressure was low immediately after inhalation of amyl nitrite showed that there was a tendency for a slight degree of myopia to develop at this time. This effect was not always observed, however, but if a refractive change did occur it was always about −0.50 D. If, while the blood pressure was still low, 0.2 cc. of fluid was injected into the vitreous, there was now either no accompanying refractive change, or a change in the direction of hypermetropia of no more than +0.50 D (10 observations). In other words, the state of general vasodilatation in the body diminished or abolished the usual hypermetropic effect of injection of fluid into the eyeball. This nullifying action was transient, lasting only as long as the

blood pressure remained low, since in one animal, which had shown no refractive change on injection of fluid into the vitreous during the time the blood pressure was low, injection of fluid into this same eye as soon as the blood pressure had risen to normal produced +0.75 D of hypermetropia, in spite of the globe having been previously punctured.

Intravenous injection of 0.2 cc. of a 1:1000 solution of adrenaline, on the contrary, caused a refractive change of as much as 2.50 D in the direction of hypermetropia along with an elevation of blood pressure. If, while the blood pressure was high, fluid was injected into the vitreous, there was still further refractive change of +3.00 D to 4.00 D in the same direction (5 observations). Still greater changes up to +18.00 D were observed with more massive doses of adrenaline (2 observations). In other words, during the state of general vasoconstriction, the effect of the injection of fluid into the eyeball was markedly enhanced.

A similar augmentation of the effect of injection of fluid into the eyeball was observed on stimulation of the cervical sympathetic nerve in the neck. The results of one experiment are given in table 2.

TABLE 2

	REFRACTION AT BEGINNING OF EXPERIMENT	REFRACTIVE CHANGE ON STIMULATION OF CORRESPONDING SYMPATHETIC NERVE	0.2 CC. SALINE INJECTED INTO R. EYE ONLY; REFRACTIVE CHANGE	REFRACTIVE CHANGE ON STIMULATION OF CORRESPONDING SYMPATHETIC NERVE	REFRACTIVE CHANGE ON INTRAVENOUS INJECTION OF 3 CC. 1:1000 ADRENALINE
R. eye	+1.00 D	+2.50 D	+2.50 D	about +18.00 D	about +18.00 D
L. eye	+1.00 D	+2.50 D		+ 2.50 D	+ 2.50 D

All these observations are in accord with a theory for the mode of action of the cervical sympathetic recently described by one of us (7), viz., that changes in tension on the suspensory ligament, and therefore the shape of the lens, are related to changes in the volume of the ciliary body, which in turn depends upon the amount of blood present in this organ, one of the most vascular st uctures in the body. The theory postulates that constriction of the blood vessels in the ciliary body so diminishes the volume of this structure that the ring of tissue shrinks back toward the sclera and thus helps to increase the tension on the suspensory ligaments and flatten the lens. When the vessels of the ciliary body are engorged and its volume increased, the tension on the suspensory ligaments is diminished, and the lens tends to round out. It has been pointed out that this theory is an application to the ciliary body of the theory of action of the iris in producing dilatation and constriction of the pupil proposed by Langworthy (8) for which he has brought forward many convincing arguments.

SUMMARY

Injection of small amounts of solutions of various tonicities into the vitreous of the cat's (or dog's) eye does not cause the appearance of cataract, but pro-

duces a transient state of hypermetropia. The effect is greatly diminished during general vasodilatation induced by inhalation of amyl nitrite, and increased during general vasoconstriction induced by intravenous injection of adrenaline, and during cervical sympathetic nerve stimulation. These results are consistent with the theory that accommodative changes in the eye are influenced by changes in the volume of the ciliary body consequent upon changes in its blood content.

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ELECTRICAL CHARACTERISTICS OF INJURIES TO HEART MUSCLE[1]

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It has been shown that a region of localized injury on heart muscle is electronegative with respect to resting uninjured muscle when the muscle is at rest and becomes electropositive with respect to the same reference when the surrounding muscle goes into activity (1). It has further been shown that the potential changes resulting are sharply localized in the region of injury or the immediate contiguous tissue (2). It was postulated that these potentials arise as the result of concentric rings of charges which reverse their polarity when the surrounding muscle goes from an inactive to an active state (1). The present study represents an attempt to determine some of the electric characteristics of this process by the application of simple electrophysical laws.

If the region of injury maintains electrical charges as implied above, it should show the following relations. If E_0 represents the potential with infinite short circuiting resistance, E_1 the potential developed with a finite short circuiting resistance $= R_1$, then the current flow is $I_1 = \frac{E_1}{R_1}$ and the impedance is $r = \frac{E_0 - E_1}{I_1} = R_1 \frac{E_0 - E_1}{E_1}$.

These relations, which are applications of Ohm's law, are commonly used in the determination of the internal resistance or impedance of batteries. It is to be noted that they give no information regarding the nature of the impedance but only its absolute value. They have been previously applied in at least one physiological study, that of the estimation of impedance in single fibers of skeletal muscle (3).

Methods. The experiments have been carried out on the ventricles in situ of large specimens of the snapping turtle (Chelydra serpentina) and on the exposed ventricles of the dog's heart. Injuries were produced in all cases by suction applied by means of a vacuum pump to a region of the surface of 1.5 or 3.0 mm. in diameter. This method of injury, first used by H. C. Wiggers (4), is superior in our experience to any other method, in that potential curves are obtained which are or approximate closely a simple monophasic curve of reproducible contour. In previous work we have employed a glass T tube with a zinc-zinc sulphate electrode provided with a wick to make contact with the heart inside the tube. It soon became apparent however that this type of electrode polarized under the relatively large current flows that are obtained when the circuit is

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loaded with a relatively low resistance. Glass tubes of approximately 1.5 cm. in diameter were drawn out to a terminal internal diameter of 1.5 or 3.0 mm. Discs of platinum of about 1 cm. diameter and continuous with a strip of the same material several centimeters in length were platinized and sealed in the tubes by means of a rubber stopper and cement. A glass tube through the stopper provided for connection to a vacuum pump to produce the suction injury. The electrode was filled with Ringer's solution sufficient to cover the disc and placed on the heart surface. The large conducting surface of the electrodes reduced current density and electrode polarization to the point that the latter did not occur until the resistance across the input of the amplifier was of the order of 100 ohms or less. Polarization of the electrode when it occurred was easily recognized by distortion of the curve.

The platinum electrode applied to the heart was paired with a plate of platinized platinum applied to the pelvis or hind leg. The electrodes led to the input of a 4 stage direct coupled amplifier and cathode ray oscillograph. The amplifier

TABLE 1

Effect of decreasing the value of R_1 (loading resistance) on maximum current flow and impedance in a typical experiment (turtle)

LOADING RESISTANCE IN OHMS	E_0 IN 10^{-3} VOLTS	E_1 IN 10^{-3} VOLTS	I_1 IN 10^{-6} AMPERES	r IN 10^3 OHMS
10^6	45.5			
5×10^3		29.50	5.88	2.72
2×10^3		17.60	8.80	3.18
10^3		10.70	10.70	3.25
5×10^2		5.27	10.50	3.83
10^2		1.05	10.50	4.23

input had a resistance of a million ohms across the leads. This we have regarded as "infinite" resistance or "zero" load. By a switching arrangement any desired lower resistance, usually of the order of two thousand ohms, could be placed across the input. This was the "loading" resistance or the factor R_1 and by its insertion the potential recorded was reduced from E_0 to E_1. All records were calibrated in millivolts and the determination of the values E_0 and E_1 gave the basis for the calculation of I_1 and r. Measurements of the curves were made with a comparator provided with micrometers and 40 × magnification (5).

Results. *The effects of decreasing the value of R_1 ("loading resistance").* As the load is increased by decreasing the loading resistance the maximum current flow rises within limits and then tends to remain constant or decrease slightly. The impedance rises continuously with increase in load. A typical example concerned with the maximum injury potential from a turtle's heart is shown in table 1.

After the data given in table 1 were obtained the electrode was removed from the heart and placed in body fluid near the heart. The impedance of the electrode system not including heart muscle was 2.27×10^3 ohms.

The voltage, maximum current flow and impedance during the negative and during

the positive phases of the injury potential. The suction electrode was placed on the heart and suction carried out while the record was being made. Normal unipolar action potentials were thus secured before the region was subjected to injury. The level of potential of resting heart muscle before injury was produced was continued into the injury curve and made possible an examination of these two phases. The first phase is the change in potential from the electronegative state during rest to the level of the reference potential. The second phase includes the change of potential from the reference level to the maximum electropositive state at the injury potential peak. The results from the statistical analysis of 54 determinations made in 7 turtles are given in table 2. It is apparent that the potential developed and the maximum current flow are significantly greater while the impedance is lower during the electropositive than during the electronegative phase.

Changes in potential, current flow and impedance with time. Figure 1 shows the potential, current flow and impedance of an injury on the ventricle of a turtle during the period when the muscle surrounding it is in activity. The data for

TABLE 2

Potential, current flow and impedance during the electro-negative and during the electro-positive phases of cardiac injuries (turtle)

	ELECTRO-NEGATIVE PHASE N = 54			ELECTRO-POSITIVE PHASE N = 54		
	E_0 in 10^{-3} volts	I_1 in 10^{-6} amperes	r in 10^3 ohms	E_0 in 10^{-3} volts	I_1 in 10^{-6} amperes	r in 10^3 ohms
Means	21.35	3.83	4.00	30.25	8.55	1.59
Standard error of means	±0.74	±0.16	±0.30	±0.78	±0.26	±0.07
Standard deviation	±5.44	±1.21	±2.23	±5.72	±1.88	±0.50
Coefficient of variation	25.4	31.6	55.6	18.8	22.0	31.4

this figure were obtained by dividing the injury potential curve into 105 arbitrary time intervals and measuring at each interval the values of E_0 and E_1. The potential of the injured region rises rapidly from a negative value of 25 mvg. to approximately the same positive value. The current at the start of the potential change is approximately 10 microamperes and falls rapidly to zero. It rises rapidly to approximately its original level as the potential reaches its maximum positive value and then declines at first gradually and later more rapidly to again reach zero as the potential changes from a positive to a negative value. The impedance is indeterminate when the current flow is zero and the curve of impedance shows two points of discontinuity. Following the first of these it is of low value and rises at first slowly and then more rapidly to reach a high value as the current falls toward zero. This rapid rise in impedance appears to be in large part responsible for the rapid fall in current during this period. Following the second reversal of potential the impedance is relatively low and is maintained nearly constant throughout the period of resting injury potential. This part of the cycle, because of the constancy of the variables during this period, has not been included in the figure.

We have made charts in five other instances from similar material with similar results, the curves differing from those reproduced here in only minor details.

The effect of size of injury on current flow and impedance. The results from 51 injuries with an electrode of 1.5 mm. aperture as compared with 54 injuries with an electrode of 3.0 mm. aperture are given in table 3. There is insignificant difference in mean voltage in the two series while the current flow is significantly greater and the impedance significantly less with the large area of injury. The loading resistance used in all cases was 2×10^3 ohms.

Comparison of injuries singly and in parallel connection. These experiments were carried out in the following manner. Two suction electrodes of the type described were used to produce two injuries simultaneously. Each was recorded singly unloaded ($R = 10^6$ ohms) and loaded ($R_1 = 2 \times 10^3$ ohms) and then by a switching arrangement the two injuries were placed in parallel and curves made unloaded and loaded. An example of the curves obtained by this procedure is given in figure 2. The two upper curves are potential time records of the two single injuries before and after applying the loading resistance. The lowest curves are similar records with the two injuries in parallel. The statistical analysis of the results from 68 single and 34 parallel injuries in the turtle and from 68 single and 34 parallel injuries in the dog is given in tables 4a and b respectively. In both it appears that with the injuries in parallel the voltages developed are not significantly different, while there is increased current flow and lower impedance with injuries in parallel than in the single injuries recorded separately.

TABLE 3

Effect of size of injury on current flow and impedance of the combined electro-negative and electro-positive phases of the injury (turtle)

	INJURY 1.5 MM. IN DIAMETER N = 51			INJURY 3 MM. IN DIAMETER N = 54		
	E_0 in 10^{-3} volts	I_1 in 10^{-6} amperes	r in 10^3 ohms	E_0 in 10^{-3} volts	I_1 in 10^{-6} amperes	r in 10^3 ohms
Means	49.68	9.71	3.14	51.62	12.38	2.19
Standard error of means	±1.10	±0.22	±0.09	±1.13	±0.32	±0.09
Standard deviation	±7.87	±1.59	±0.65	±8.28	±2.35	±0.37
Coefficient of variation	15.7	16.3	20.6	16.0	19.0	16.9

Potential, current flow and impedance during the QRS period of action potentials. Data from unipolar action potentials recorded with the electrode of 1.5 and 3 mm. diameter are summarized in table 5. These data were derived by determining the total voltage change during the QRS period without load ($R = 10^6$ ohms) and after loading ($R_1 = 2 \times 10^3$ ohms) in the usual manner, the suction electrode being used before suction was applied. After a few normal cycles suction was applied and the data on injury potentials obtained which are summarized in table 3.

Discussion. Although in cardiac injuries produced by suction, monophasic potential-time curves of similar contour are consistently obtained they may differ in total voltage, relative time of onset, and are subject to the usual variations

common to any physiological experiment. Because of this it early became apparent that valid conclusions could be drawn only from a relatively large amount of data analyzed statistically. In addition to the data presented in the tables, total correlations were made in all cases between the variables and the significance of differences between means tested by use of the critical ratio. Correlations between E_0, E_1, I_1 and r were in all cases such that the probability of the correlation being due to chance was less than one in a hundred ($P < 0.01$) except as noted below. As expected the correlations between E_0, E_1 and I_1 were positive, those between I_1 and r negative. Two correlations, both involving I and r, one in parallel injuries in the turtle and the other in parallel injuries in the dog, had levels of significance of $P > 0.01 < 0.05$. The determination of the

TABLE 4

Potential, current flow and impedance of injuries in single and parallel connections

	SINGLE INJURIES N = 68			INJURIES IN PARALLEL N = 34			
	E_0 in 10^{-3} volts	I_1 in 10^{-6} amperes	r in 10^3 ohms	E_0 in 10^{-3} volts	I_1 in 10^{-6} amperes	r in 10^3 ohms Observed	Calculated
a. Turtle							
Means	48.27	7.60	4.52	44.93	10.27	2.40	2.22
Standard error of means	±1.50	±0.32	±0.09	±1.79	±0.44	±0.06	±0.05
Standard deviations	±12.38	±2.62	±0.75	±10.43	±2.57	±0.37	±0.28
Coefficient of variation	25.6	34.5	16.5	23.2	25.0	15.4	13.2
b. Dog							
Means	75.84	18.29	2.20	75.87	24.22	1.15	1.09
Standard error of means	±1.63	±0.44	±0.51	±2.03	±0.63	±0.04	±0.04
Standard deviations	±13.41	±3.63	±0.48	±11.81	±3.65	±0.22	±0.21
Coefficient of variation	17.7	14.4	21.8	15.5	15.0	19.0	19.2

significance of difference between means involved the means of E_0, I_1 and r with the electrodes of 1.5 and 3.0 mm. and between the same means in single and parallel connections in the turtle and the dog. The mean of the E_0's differed by an amount which could be accounted for by chance at the probability level of 0.16 to 0.99 and these differences are regarded as insignificant. The difference between the means of the I_1's and r's were all above $P < 0.0000$ and are regarded as significant. The results obtained by statistical analysis of the data are in agreement with the postulates made in reference to the nature of the electrical phenomena of an injured region.

The maximal potential developed and maximal current flow of comparable ventricular injuries in turtles and dogs are considerably less in the former animal, while the impedance in the turtle is higher than in the dog (tables 4a and b).

The impedance as determined represents the impedance between the two electrodes, including the impedance of the heart and the impedance of the suction electrode. The suction electrodes used were all of the same pattern and differed only as to the area of contact with the heart muscle. The impedance of the suction electrode itself is small, less than 100 ohms, and the impedance of the body between the electrodes, exclusive of the heart, may be regarded as relatively constant during the course of an experiment. The principal variable factor in regard to impedance in these experiments is in the heart.

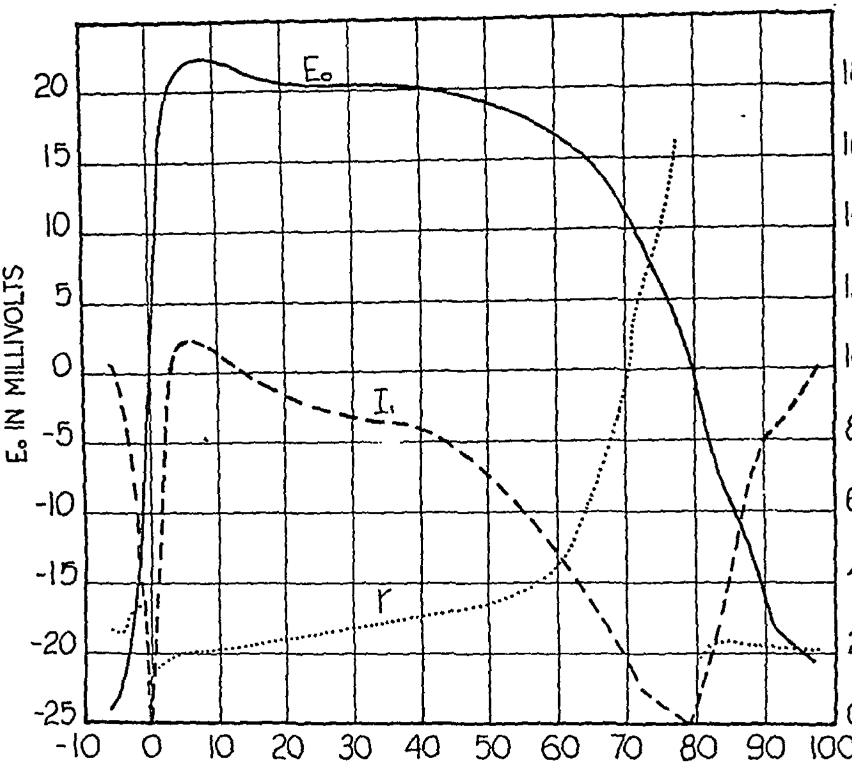

Fig. 1. Voltage, current flow and impedance changes in an injured region on the ventricle of a turtle during the systolic period. Description in text.

In measurements of impedance in heart muscle by the use of an outside source of power in the form of an alternating current applied through a bridge network, it was recognized that contraction of the muscle by increasing its thickness may of itself increase the impedance. In order to avoid this workers using this method (6, 7) have made their measurements with preparations contracting isometrically, either ventricular strips or the whole ventricle. The impedance in the present work is determined by using the heart itself as the power source without the application of any outside applied power and the intact heart in situ contracted in the normal isotonic manner. Changes in impedance which may be in part due to alterations in thickness of the muscle are those which occur in the heart contracting in a normal manner. All of our determinations of impedance, except those made throughout the systolic period, as illustrated in figure 1, have however

been made at comparable periods in the contraction. It has previously been shown that the injury potential-time curve from any region of heart muscle bears a close relation to the onset of contraction in contiguous regions of normal muscle (8). Contraction begins at or near the point where the injury potential changes from a negative to a positive value. The results summarized in table 2 were derived from measurements made at two periods, the maximum potential change

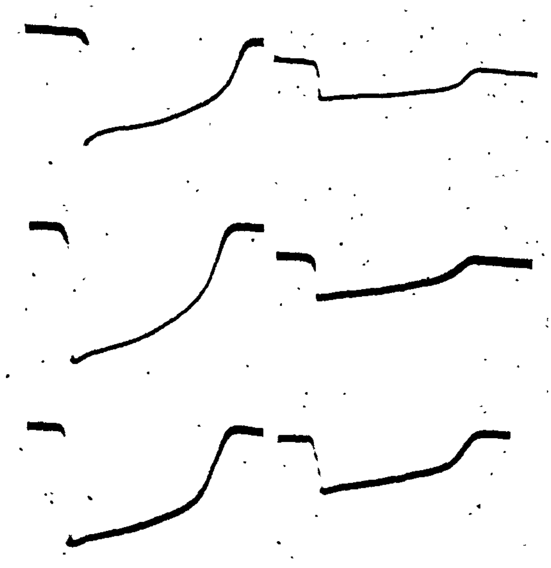

Fig. 2. Potential-time curves from two regions of injury on the ventricle of a turtle, recorded singly and in parallel. Further description in the text.

TABLE 5

Action potentials (unipolar leads) of turtle ventricle

	ELECTRODE 1.5 MM. IN DIAMETER N = 51			ELECTRODE 3.0 MM. IN DIAMETER N = 54		
	E_0 in 10^{-3} volts	I_1 in 10^{-6} amperes	r in 10^3 ohms	E_0 in 10^{-3} volts	I_1 in 10^{-6} amperes	r in 10^3 ohms
Means	12.52	2.03	5.12	13.58	3.16	2.51
Standard error of means	±0.51	±0.13	±0.45	±0.61	±0.17	±0.19
Standard deviation	±3.60	±0.91	±3.23	±4.48	±1.27	±1.37
Coefficient of variation	23.8	44.7	63.0	33.0	40.0	55.0

preceding and the maximum potential change following the point of potential reversal. In all other instances the total potential change from its maximum negative to its maximum positive value was used for the calculation of the impedance. Action potentials in general show much greater variations than do injury potentials as is evidenced by the larger coefficients of variation in the former (tables 3 and 5). However, as in injury potentials, the use of the large electrode is associated with significantly greater current flow and diminished impedance as compared with the smaller electrode. Significant correlation co-

efficients are also obtained between the variables E_0, and I_1 and r. On comparing action and injury potentials from the same region (tables 3 and 5) it is obvious that the voltage developed and the maximum current flow is greater and the impedance less in the injury potentials than in the action potentials. Attempts to determine whether any correlation exists between the voltage, current and resistance in the action potential and in the injury potential from the same region led to uncertain results. Correlations between the two using the 3 mm. electrode were significant at the 0.05 to 0.01 level. However, correlations between the two using the 1.5 mm. electrodes were entirely insignificant.

SUMMARY

Measurements of voltage, current flow and impedance have been made from injured regions of ventricular muscle of the turtle and dog and during the QRS period of the normal unipolar action potential of the turtle. The method used was to record potential time curves with high resistance across the input leads to the amplifier followed by similar curves with relatively low resistance across the input (loading resistance). The values of the voltages recorded served for the calculation of current flow and impedance. With decreasing values of resistances across the input to the amplifier, the maximum current from injuries increases up to a certain point and then tends to remain nearly constant. The impedance increases continually with loading resistances from 5000 to 200 ohms. During the period when the heart muscle surrounding an injury is in activity, changes in voltage, current flow and impedance occur which are described in the text. Increasing the area of injury results in increase in the maximum current flow and decrease in impedance. The same results are obtained in recording two injuries in parallel connections as compared with recording each alone. The results are in agreement with the postulation that an injured region of heart muscle maintains electrical charges which undergo variations in amplitude and polarity when the muscle surrounding the injury enters into activity.

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THE EFFECT OF ADRENALECTOMY, ADRENAL CORTICAL HORMONES, AND TESTOSTERONE PROPIONATE PLUS ADRENAL CORTICAL EXTRACT ON THE "ALKALINE" AND "ACID" PHOSPHATASES OF THE LIVER AND KIDNEY OF THE RAT[1,2]

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In a recent report (3) it was demonstrated that the stimulation by adrenal cortical extract of glyconeogenesis from protein in the adrenalectomized rat was not accompanied by an increase in the arginase activity of the liver, but that of the kidney was partly restored to normal. Previous treatment with testosterone propionate did not alter the effects of the adrenal cortical extract but greatly increased the kidney arginase.

The above study has been extended to two other enzymes, the "alkaline" and "acid" phosphatases.

METHOD. *Adrenalectomy.* White rats of the Wistar strain were adrenalectomized under ether anesthesia, in groups of 8 to 12.

Hormone preparations. Doses of 2.5 mgm. of testosterone propionate,[4] in 0.1 ml. of sesame oil, were injected in the morning and the evening from the day of operation, including the morning of the day of the autopsy.

The desoxycorticosterone acetate[4] was injected at 1 mgm. per day from the day of the operation.

The adrenal cortex extract (aqueous, Upjohn)[5] was concentrated just before use in vacuo at 30–40° to about one-half its volume in order to remove the alcohol; the volume was restored with water, except in the 2 ml. per injection experiments, when it was made to exactly one-half of its original volume. The injections were given on the 5th postoperative day at hourly intervals for 8 hours (4).

PROCEDURE. The rats were placed in individual cages as a rule 3 days before the operation and were given 12 grams of Purina fox chow meal and 1 per cent sodium chloride or tap water at 5 p.m. each day. No food was given on the 4th post operative day and the drinking tubes were removed (5) on the next morning

[1] This investigation was aided by grants from the Josiah Macy Jr. Foundation.

[2] The data contained in a preliminary note (1) are not included in this report. Parts of these data have been reported in the Josiah Macy Jr. Foundation Conference on Metabolic Aspects of Convalescence. Eighth meeting 50 (1944) and tenth meeting 68, (1945), and in a review (2).

[3] The data in this paper were taken from a thesis submitted by Virginia N. Vail to the Graduate School, the University of Rochester, in partial fulfillment of the requirements for the M.S. Degree, July 1945.

[4] The testosterone propionate (perandren) and the desoxycorticosterone acetate (percorten) were provided by Ciba Pharmaceutical Products, Inc.

[5] Part of this material was provided by Dr. M. Kuizenga, The Upjohn Company, and the rest was purchased from local dealers.

just before the injections of the adrenal cortical extract were begun. One hour after the last injection of the adrenal cortical extract, the rats were injected intraperitoneally with sodium amytal at 9 mgm. per 100 grams of body weight. When anesthesia was complete the liver was rapidly removed, the left section of the median lobe cut off and the remainder was weighed and analysed for glycogen (6, 7). A piece, 1–2 mm. thick, was removed from the left section of the median lobe and placed in 15–20 ml. of absolute redistilled acetone for the histochemical determination of the "alkaline" phosphatase (8). Another piece was removed for the determination of water content and the remainder was weighed and placed in 5 ml. of cold redistilled water.

The right kidney was removed, weighed and placed in 5 ml. of cold redistilled water. The left kidney was removed, weighed and a 1–2 mm. thick cross section was removed and placed in acetone for the histochemical enzyme studies.

Enzyme determinations. The tissues were homogenized (9, 10) and enough water was added to give a concentration of 20 ml. water per gram of tissue. These stock homogenates were diluted 4 fold for the "acid" phosphatase determination and the kidney sample was diluted 30 fold for the "alkaline" phosphatase determination. The "alkaline" phosphatase was determined at pH 9.8 by the King-Armstrong method (11) with minor modifications (10) and the "acid" phosphatase at pH 5.4 (10) with the Robinson-Gutman substrate (12).

Histochemical determination of "alkaline" phosphatase.[6] The method of Gomori (8) without any counterstaining was used for the histochemical determination of the "alkaline" phosphatase. The enzyme activity of the liver was enhanced by the addition of 10 parts of M/10 Mg SO_4 (13, 14) to the incubation mixture.

Nitrogen determination. Nitrogen was determined by analysing aliquots of the tissue homogenates by the micro-Kjeldahl method.

Water content. The piece of liver was dried to constant weight at 90–100° in an electric oven.

Results. *Weight and composition of the liver.* Adrenalectomy produced the expected decrease in glycogen and no remarkable changes in the weight, water and nitrogen (protein) content of the liver (table 1). The administration of water instead of 1 per cent sodium chloride or daily injections of desoxycorticosterone acetate did not influence the effects of the adrenalectomy. The results after the sham operation are essentially identical to those obtained in the normal rats.

The administration of adrenal cortical extract had no remarkable effect on the weight or water content of the liver. It did produce, however, a small but significant decrease in the nitrogen (protein content) and the expected increase in glycogen (table 1). Previous treatment with testosterone propionate slightly decreased the effects of the adrenal cortical extract on the liver glycogen. The changes, however, were small and not significant.

Liver phosphatases. Adrenalectomy slightly increased the "alkaline" phosphatase of the liver (table 2). Desoxycorticosterone acetate had no influence

[6] Charles Luttrell assisted in carrying out some of these determinations and in the study of the prepared histological sections.

but the adrenal cortical extract produced a very large increase in the enzyme. The livers of the adrenalectomized animals possessed small amounts of the enzyme which was localized (black areas) in the cells at the periphery of the lobules (fig. 1). After the administration of the adrenal cortical extract the amount present in this area increased and also the enzyme appeared (was produced?) in

TABLE 1

The effect of adrenalectomy, adrenal cortical hormones and testosterone propionate (T.P.) plus adrenal cortical extract (A.C.E.) on the composition of the liver

	NO.	BODY WGT.	WEIGHT	GLYCOGEN	WATER	NITROGEN
		gm.	*per cent**	*per cent**	*per cent**	*per cent**
Water ad libitum	8	144	0	+8	0.0	−0.5
Desoxycorticosterone acetate, 1 mgm/day	6	161	−5	+9	−1.0	−1.4
Normal	8	145	+5	+470	−2.6	+1.4
Sham operation	4	150	+5	+465	−2.2	+2.5
A.C.E., 8 × ½ ml/hr	13	157	+9	+560	−0.7	−4.1
A.C.E., 8 × 1 ml/hr	3	154	−1	+1750	−2.5	−3.8
A.C.E., 8 × 2 ml/hr	6	142	+10	+1370	0.0	−7.4
T.P., 2 × 2.5 mgm/day + A.C.E., 8 × ½ ml/hr	11	158	+11	+375	−1.5	−3.3
T.P., 2 × 2.5 mgm/day + A.C.E., 8 × 2 ml/hr	6	150	−1	+1200	−0.9	−5.5

* Change from average values of 10 control rats: Body weight 156 grams; weight 3.34 grams/100 grams body weight; glycogen 65 ± 18 mgm/100 grams liver; water 72.5 ± 1.3 grams/100 grams liver and nitrogen 3.66 ± 0.11 grams/100 grams liver.

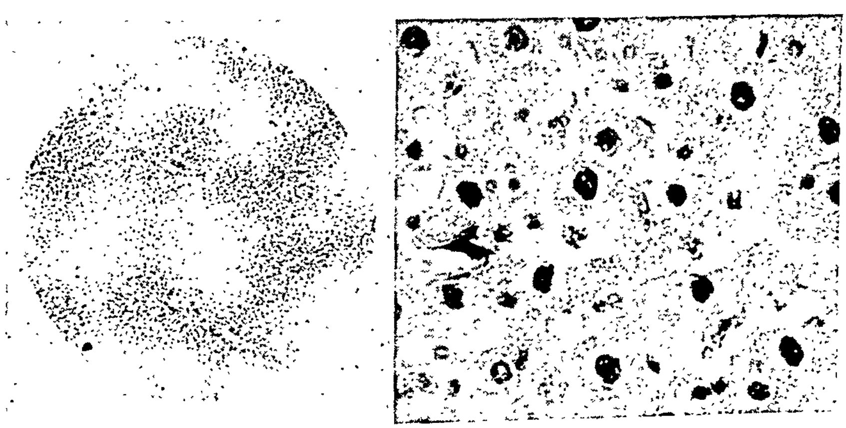

Fig. 1. The "alkaline" phosphatase (black areas) of the liver of an adrenalectomized rat receiving 0.9 per cent sodium chloride as drinking water. A(25×) B(200×)

relatively large concentration in the walls, cytoplasm and nuclei of all of the other cells (fig. 2). The increase in the "alkaline" phosphatase was immediate (fig. 3). There was, at first, a spurt in the production of the enzyme followed by a slight decline and a gradual increase with increase in glycogen formation.

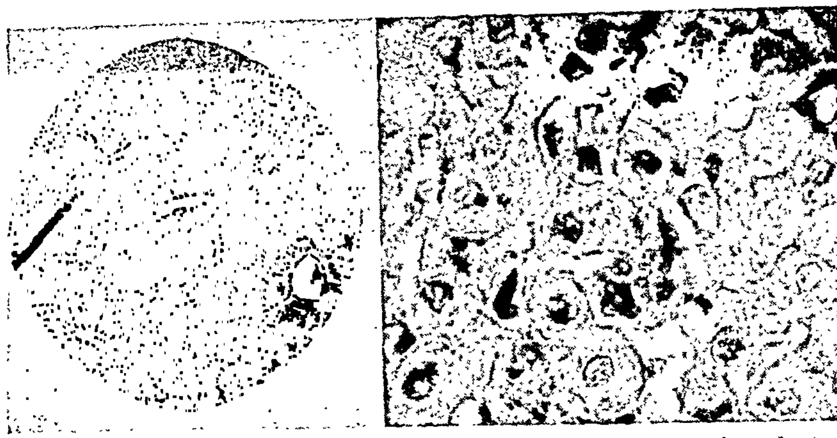

Fig. 2. The "alkaline" phosphatase (black areas) of the liver of an adrenalectomized rat receiving 0.9 per cent sodium chloride as drinking water, daily injections of testosterone propionate and on the last day adrenal cortical extract (aqueous Upjohn). A(25×) B(200×)

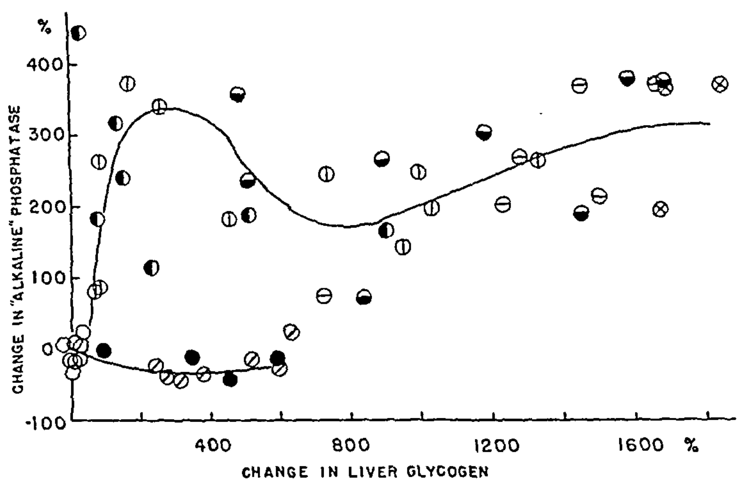

Fig. 3. The effect of the adrenal cortex on liver glycogen and "alkaline" phosphatase. The values are plotted as differences from those of adrenalectomized rats which received 1 per cent Na Cl as drinking solution. The per cent changes are for the milligrams glycogen per 100 grams and units of enzyme per gram of liver.

Normal ⊘; sham operation ●; adrenalectomized rats receiving water instead of 1 per cent Na Cl, ○; adrenal cortical extract, $\frac{1}{2}$ ml. hr., ⦶; 2 × 2.5 mgm. testosterone propionate per day plus adrenal cortical extract, $\frac{1}{2}$ ml. hr, ◐; adrenal cortical extract 1 ml. hr., ⊗; adrenal cortical extract, 2 ml. hr., ⊖; 2 × 2.5 mgm. testosterone propionate plus adrenal cortical extract, 2 ml. hr., ◒. The values for the rats treated with desoxycorticosterone acetate are omitted because they were the same as those of the rats receiving water and the lack of space did not permit their insertion.

Note the sharp increase in the enzyme activity of the liver of the treated rats and the lack of increase in the normal rats.

Previous treatment with testosterone propionate had no effect on the enzyme response obtained with the adrenal cortical extract.

The livers of normal animals with amounts of glycogen similar to those of adrenalectomized rats treated with adrenal cortical extract showed a slight decrease rather than an increase in the "alkaline" phosphatase (table 2, fig. 3).

The "acid" phosphatase was not altered by any of the above treatments, therefore the data on this enzyme are omitted.

Kidney phosphatases. The administration of sodium chloride as drinking water or daily injections of desoxycorticosterone acetate prevented the small decrease in the "alkaline" phosphatase of the kidney noted after adrenalectomy. The intensive treatment with the adrenal cortical extract produced variable and questionable changes. Testosterone propionate, on the other hand, produced a very significant increase in the enzyme. The histochemical studies confirmed the results obtained with the homogenates. Furthermore, there were no observable alterations in the histological distribution of the enzyme as a result of the various treatments.

TABLE 2

The effect of adrenalectomy, adrenal cortical hormones and testosterone propionate (T.P.) plus adrenal cortical extract (A.C.E.) on the phosphatases of the liver and kidney

	NO.	LIVER		KIDNEY PHOSPHATASES	
		Weight	"Alkaline" phosphatase	"Acid"	"Alkaline"
		grams	*per cent**	*per cent**	*per cent**
Water ad libitum	8	4.835	−5	+2	−23
Desoxycorticosterone acetate 1 mgm/day	6	5.112	−8	+2	+9
Normal	8	5.078	−17	+15	−15
Sham operation	4	5.255	−19		−1
A.C.E., 8 × ½ ml/hr	13	5.704	+225	0	+3
A.C.E., 8 × 1 ml/hr	3	5.058	+303		−12
A.C.E., 8 × 2 ml/hr	6	5.197	+246	+2	−19
T.P., 2 × 2.5 mgm/day + A.C.E., 8 × ½ ml/hr	11	5.484	+208	−2	+36
T.P., 2 × 2.5 mgm/day + A.C.E., 8 × 2 ml/hr	6	4.968	+276	+2	+36

* Change from average values of 10 control rats: Liver "alkaline" phosphatase, 3.6 ± 0.73 units/gram; kidney "acid" phosphatase, 20.5 ± 2.4 units/grams; "alkaline" phosphatase 221 ± 67 units/grams.

The small decrease in "acid" phosphatase after adrenalectomy is of questionable significance since in the first series of experiments (1) the enzyme was restored to normal by the adrenal cortical extract but in this series of experiments (table 2) it was not influenced by any of the various treatments.

Discussion. The slight inhibition in the glyconeogenic properties of the adrenal cortical extract by testosterone propionate suggests that there may be a counterbalancing influence between these two hormones. The failure, however, to obtain significant effects indicates that the "S-hormone" when given in a sufficiently high dose will overpower the previously established anabolic effect of the "N-hormone". This is not too surprising. It would be of interest to set

up a chronic type of experiment in which the adrenal cortical extract was administered at a dose sufficient for maintenance of glyconeogenesis at a normal level and then superimpose a potent "N-hormone".

The marked increase in "alkaline" phosphatase of the liver by adrenal cortical extract indicates that this enzyme is involved in the glyconeogenic effect of the C_{11} adrenal cortical steroids. Although it is impossible with our present knowledge of this enzyme to indicate precisely what phase of the process is concerned, it is nevertheless well known that carbohydrate metabolism proceeds through phosphorylated intermediates (15) and that high "alkaline" phosphatase activity occurs at sites of accelerated protein metabolism, e.g., healing wounds (16), bone (17), in the silk glands of the caterpillar, of the goat-moth and the common spider (18) and in the skin of mice undergoing carcinogenesis by applications of methylcholanthrene (19). On the other hand the formation of liver glycogen from dietary protein or carbohydrate is not accompanied by increases in the enzyme comparable to that induced by the adrenal cortical extract (unpublished).

The presence of similar amounts of glycogen but not "alkaline" phosphatase in the livers of adrenalectomized rats receiving the lowest dose of adrenal cortical extract and normal rats is due probably to the different metabolic status of these animals. The glycogen in the liver of the injected rat was being newly and rapidly formed from protein reserves while that of the normal rat was a residuum which was in the process of being expended.

The maintenance of normal "alkaline" and "acid" phosphatases in the liver after adrenalectomy is of special interest since the ability to deaminate glutamic acid and alanine (20) also is not affected, but oxidative processes (21) as noted by O_2 consumption and arginase activity (3, 22, 23) are decreased.

The failure to observe a marked decrease in the "alkaline" phosphatase of the kidney after adrenalectomy is very likely due to the relatively short interval, 5 days, between operation and determination of enzyme activity for Folley and Greenbaum (22) obtained a great decrease in the activity of this enzyme in the kidney of the lactating rat 20 days after operation. Furthermore, there was a greater decrease (about 70 per cent) in the rats receiving a high (50 per cent) protein diet than (about 40 per cent) in those receiving the stock colony diet which contained 20 per cent protein. Similar decreases had been previously observed by Kutscher and West (24) in the guinea pig and by Jiminez-Diaz (25) in the cat.

The prevention of the small decrease in the enzyme of the kidney by the administration of desoxycorticosterone acetate is in agreement with the observations of Folley and Greenbaum in the lactating rat (22) and Kutscher and Wust in the guinea pig (24). It is worthy of note that desoxycorticosterone acetate, injected at 3 mgm/day was more effective than adrenal cortical extract, 11-dehydrocorticosterone or 11-dehydro-17-hydroxycorticosterone in maintaining the "alkaline" phosphatase activity of the kidney of the lactating rat (22). It is not surprising, therefore, that the acute treatment with the adrenal cortical extract in our experiments did not produce any significant increases in this enzyme. On the other hand, the very pronounced ability of testosterone pro-

pionate not only to maintain but also to increase the enzyme in the kidney of the adrenalectomized rat would suggest that this may be one of the functions of the androgenic substances produced by the adrenal cortex (26).

SUMMARY

Adrenalectomy slightly increased the "alkaline", pH 9.8, phosphatase of the liver of young (150 gram) adult male rats. The hourly injection for eight hours of adrenal cortical extract (aqueous Upjohn) on the fifth post operative day greatly increased this enzyme. The increase occurred at a much faster rate than the increase in glycogen. Histochemical studies demonstrated greater amounts of the enzyme to be present (produced?) in the cytoplasm, walls and nuclei of the liver cells. Previous treatment with testosterone propionate did not alter the effect of the adrenal cortical extract on either the amount of glycogen or the increase in the enzyme activity. Desoxycorticosterone acetate was ineffective.

Adrenalectomy resulted on the fifth post operative day in a small decrease in the "alkaline" phosphatase of the kidney which was prevented by the administration of 1 per cent sodium chloride as drinking water or by the daily injection of 1 mg. of desoxycorticosterone acetate. The hourly administration for 8 hours of adrenal cortical extract on the fifth post operative day was ineffective. Testosterone propionate, 2×2.5 mgm. day, produced a marked increase in this enzyme.

None of the above treatments produced a significant change in the "acid" phosphatase, pH 5.4, of either the liver or the kidney.

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HYPERGLYCEMIA INDUCED BY THE ACTION OF ADRENALIN ON THE CENTRAL NERVOUS SYSTEM[1]

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Becht, working on dogs (1), Leimdorfer on cats (2, 3), and Heller on cats and dogs (4), have shown that adrenalin injected intrathecally fails to raise blood pressure, although the same amount injected intravenously into the same animal produces a great rise. This has been seen in anesthetized cats and dogs, in decerebrated cats and, as indicated by the present work, in unanesthetized man. The blood pressure remains usually at the same level for many hours, or falls slightly. A significant fall in blood pressure has not been reported except by Heller who recorded a terminal fall from 88 to 14 mm. of mercury in a single cat following the second of two injections of 0.3 mgm. of adrenalin, the interval between injections being 50 minutes (4). In our experience far larger doses have not had a similar effect. In dogs, adrenalin given intrathecally produces a fall in body temperature (5), complete analgesia which may last for two hours (6), and sleep for as much as five hours (5). The respiration and reflexes remain normal.

Becht found that adrenalin was still present in the cerebrospinal fluid of the dog 5 hours after intracisternal injection (1) and we have obtained the same results in the cat.

The available literature does not report the effect of intracisternal injection of adrenalin on the concentration of glucose in the blood. It was found in the present work that an increase in blood glucose concentration occurs, and then each of the known pathways whereby the central nervous system might affect the blood glucose level was interrupted surgically.

EXPERIMENTAL. The investigations were performed on 71 cats, 2 dogs, and 2 human beings. Blood pressure was determined in cats and dogs anesthetized with dial-urethane[2] by means of a mercury manometer connected to a cannula in the femoral artery, heparin or chlorazol-fast pink being used as anticoagulant. In man blood pressure was determined by means of an aneroid manometer and auscultation. Electroencephalograms and electrocardiograms were recorded with a 6-channel Grass-electroencephalograph.

The animals used in experiments concerned with the blood glucose level were anesthetized mainly with sodium amytal and a few with dial-urethane. Blood glucose was determined by colorimetric measurement of ferricyanide reduction using tungstic acid filtrates (7). Commercial preparations of adrenalin, which contain bisulfite and fresh solutions of adrenalin in dilute hydrochloric acid were used.

[1] Aided by grants from the Rockefeller Foundation and the Josiah Macy, Jr. Foundation.

[2] We are indebted to Ciba for the Dial.

Injections into the cisterna magna were made after withdrawal of a volume of cisternal fluid equal to the volume of fluid to be injected. When blood was found in the cisternal fluid the experiments were discontinued. Animals in which the cervical cord had been sectioned below the emergence of the phrenic nerve or in which the hypophysis had been removed or its stalk cut were not used until enough time had elapsed for the sealing of the subarachnoid space. Adrenalectomized and vagotomized preparations were used at once or within a day. Injections of adrenalin by other routes were frequently made for comparison. Cisternal fluid withdrawn at various times after intracisternal injection of adrenalin was tested for pressor- or glucose-raising action by intravenous injection of fluid into the same or another animal.

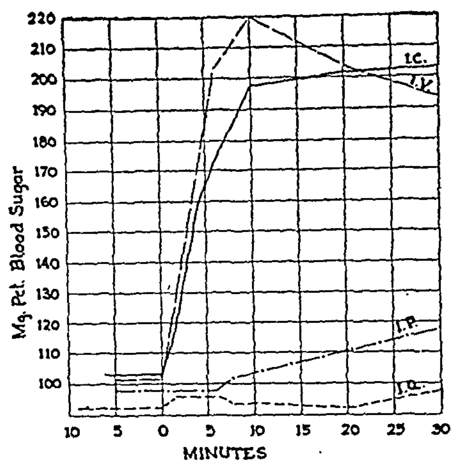


Fig. 1. Blood sugar (in mgm. per cent) after injection of 50 micrograms of adrenalin per kgm. body weight by different routes of administration at 0 time. Fasting cats. Sdoium amytal anesthesia.
The lowest curve — — — i.a. after intra-articular injection into the knee-joint of the hind limb.
Curve -·-·-·- i.p. after intraperitoneal injection.
Curve ———————— i.c. after intracisternal injection.
The uppermost curve —— —— i.v. after intravenous injection.

RESULTS. The principal finding was that injection of adrenalin into the cisterna magna was followed by a prompt, marked and sustained rise in blood glucose. Figure 1 shows (in the cat under sodium amytal) there was a rapid rise beginning 2 minutes after intracisternal injection, the concentration in blood glucose increased at a slower rate during the next several hours, as indicated in figure 1 and figure 2 A.

The increase and duration of an elevated level of blood glucose depended directly upon the dose of adrenalin. In the cat the threshold for a rise in blood glucose was 5 micrograms per kgm. This dose gave a rise of about 30 mgm. per cent at one hour after which there was a slow decline during approximately six hours. Figure 2 B illustrates that with large doses the increase in blood glucose could be maintained for more than 18 hours. This suggests that the adrenalin in the subarachnoidal space disappears slowly.

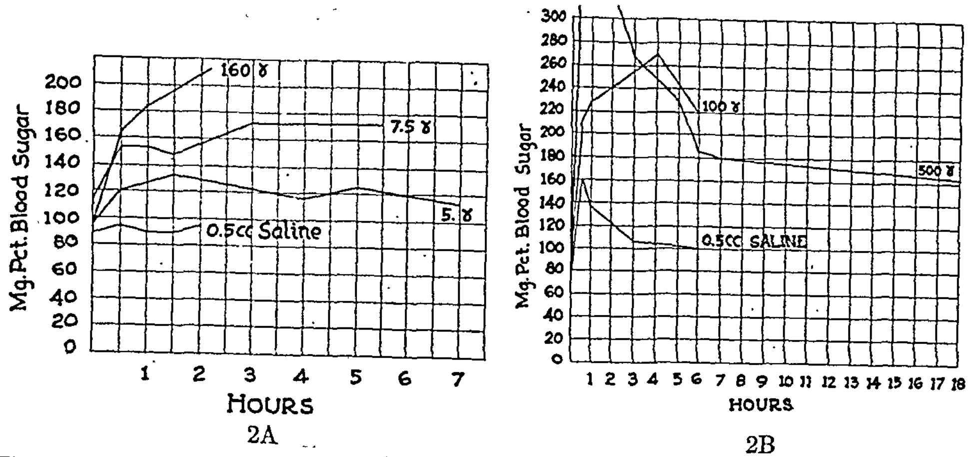


Fig. 2. A. Fasting cats. Sodium amytal anesthesia. Blood sugar after intracisternal injection of 0.5 ml. of physiological saline and of different amounts of adrenalin.

B. Fasting cats without anesthesia. Otherwise same as 2A.

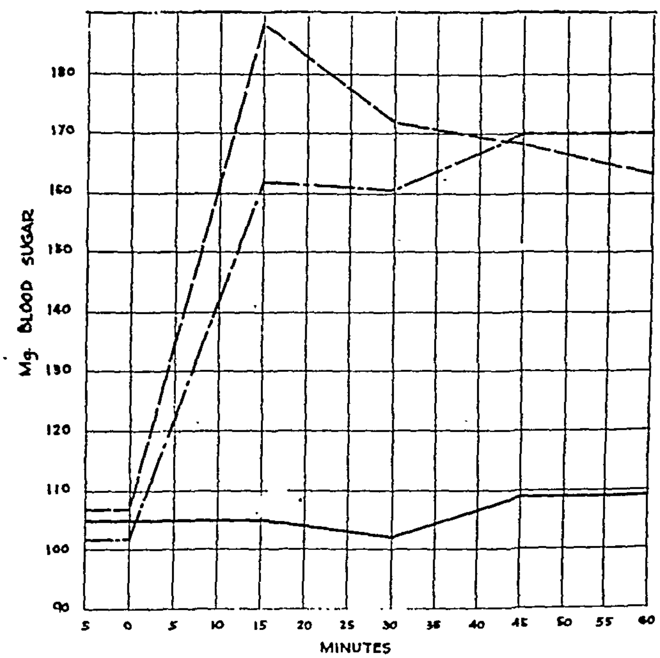


Fig. 3. Blood sugar (in mgm. per cent) after intravenous injection of cisternal fluid at 0 time under varying conditions. Fasting cats. Sodium amytal anesthesia.

Curve— — —after intravenous injection of 0.5 ml. of cisternal fluid which had been withdrawn from a second cat (5 hrs. after intracisternal injection of 0 mgm. of adrenalin).

Curve –·–·–·– after intravenous injection of 0.5 ml. of cisternal fluid withdrawn from a second cat (16 hrs. after intracisternal injection of 0.5 mgm. of adrenalin).

Curve ——— after intravenous injection of 0.5 ml. of cisternal fluid withdrawn from a second cat which had not received adrenalin.

In agreement with that assumption is the following: 1 ml. of spinal fluid, withdrawn 16 hours after intracisternal injection of 0.25 mgm. adrenal in per kgm.

when injected intravenously into a second cat caused a rise of 70 mgm. per cent glucose concentration (fig. 3). On the other hand 0.25 mgm. of adrenalin per kgm. injected intravenously could not be detected by the biological assay of cisternal fluid withdrawn one-quarter hour after this injection.

When dial was used as an anesthetic the frequently high and variable blood glucose levels tended to obscure the effect of adrenalin. However, when the pre-injection glucose level was relatively low the hyperglycemic effect was like that under amytal.

Intracisternal injection of 0.5 ml. of physiological saline provoked no rise in blood glucose in cats under anesthesia, only intracisternal saline (0.5 ml.) in cats without anesthesia was followed by a rise lasting one hour and varying in degree with excitement and struggling at the time of injection.

In contrast, the rise in blood glucose after intravenous injection of adrenalin was prompt, rapid and high, but not sustained. After intravenous injection of 50 micrograms per kgm., the concentration of blood glucose reached a peak of 220 mgm. per cent in 10 minutes and then fell steadily, reaching the level of 138 mgm. per cent in 60 minutes and the pre-injectionlevel in approximately 90 minutes. Figure 1 also shows the slow, small rise obtained by a like dose given intraperitoneally and the flat curve following an equal injection into the intra-articular cavity of the knee.

Further, 5 micrograms of adrenalin per kgm. intraperitoneally, intramuscularly or subcutaneously gave no rise in blood glucose and the same amount intravenously provoked a rise in blood glucose of about 90 mgm. per cent, but the blood glucose concentration returned to normal within one hour or two.

The incidental findings extend the range of dose of intracisternal adrenalin which had no effect on blood pressure to 5 mgm. per kgm. Further, large doses (0.5 mgm.–1.0 mgm. per kgm.) intracisternally in non-narcotized cats gave a transitory picture of wide, uncertain gait, momentary excitement and subsequent drowsiness lasting up to 18 hours. Another observation in these cats was that after intracisternal adrenalin their pupils were moderately dilated but constricted to light. Moreover, intracisternal adrenalin doubled the rate of respiration and increased its depth when the rate of respiration was low after transection of the mid-thoracic region of the spinal cord in the cat.

Intracisternal injection of 2 mgm. of adrenalin into a non-narcotized patient of 60 kgm. body weight induced a rise in blood glucose (from 112 mgm. per cent to 212 mgm.). The blood glucose concentration was elevated for more than 3 hours, but no rise in blood pressure or heart rate occurred. About 1 hour after the injection drowsiness, a little later normal sleep (confirmed by E.E.G. records) was observed in this patient.

It should be noted that in cats intracisternal injection did not produce any change in the electroencephalogram, electrocardiogram or heart rate. Intravenous injection of adrenalin or of cisternal fluid withdrawn several hours after intracisternal injection of adrenalin produced occasional irregular spike-like potentials, chiefly in parietal and occipital regions, small, fast, electrical activity of the cortex, and also extrasystoles, increased heart rate and blood pressure and some-

times a brief arrest of respiration during the rise in blood pressure. All of these effects are indicated in figures 4, 5 and 6.

Finally, no one of the operations listed below had any detectable effect on the hyperglycemic response which followed intracisternal injection of adrenalin. Each lesion was checked by autopsy: 1, almost complete hypophysectomies,

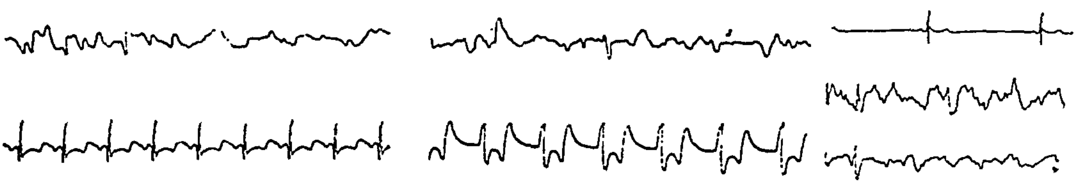
4A 4B 4C

Fig. 4. Cat. Dial anesthesia. Upper tracing—respiration, lower tracing—blood pressure. Scale represents 5 seconds per interval.

A, at *a* intracisternal injection of 0.24 mgm. of adrenalin. At *b* intracisternal injection of 0.24 mgm. of adrenalin.

B, at *a* 0.5 ml. CSF withdrawn 15 minutes after intracisternal injection of 0.48 mgm. of adrenalin. At *b* intravenous injection of *a* CSF into the same animal; rise in blood pressure from 78 mm. Hg to 197 mm. Hg.

C, at *a* same as B (above). CSF removed 30 minutes after intracisternal injection of 0.48 mgm. of adrenalin. At *b* intravenous injection of CSF *a* into the same animal; rise of blood pressure from 74 mm. Hg to 148 mm. Hg.



5A 5B 6

Fig. 5. Cat (same animal as fig. 4). Upper record electroencephalogram, lower record electrocardiogram.

A, after intracisternal injection of 0.48 mgm. of adrenalin.

B, after intravenous injection of 0.5 ml. of cisternal fluid from cat A.

Fig. 6. Cat. Dial anesthesia. Electrocardiogram (topmost record) and electroencephalogram—biparietal and bioccipital (two lower records) one minute after intravenous injection of 0.25 mgm. of adrenalin. Heart rate 48 per minute. Note the diphasic spike at the left of the figure in both electroencephalographic records.

the stalk being ablated in five cats; 2, section of the hypophyseal stalk in two cats and one dog; 3, section of the vagi at the level of the carotid sinus in one cat; 4, section of the spinal cord at C 7 in two cats; 5, ablation of the celiac ganglion in one cat; 6, adrenalectomy in two cats; 7, tying off the common carotid arteries in one cat; 8, combined hypophysectomy and vagotomy in one cat; 9, combined complete section of the spinal cord at C 7 and vagotomy in one cat.

Discussion. If adrenalin passed slowly from the subarachnoid space into the circulation, a small rise in blood pressure would be expected from the findings

of Kretschmer (8). He demonstrated in rabbits that a continuous intravenous infusion of very dilute solutions of adrenalin increased the blood pressure up to some maximum which was then maintained. Moreover, A. E. Koehler et al. reported in 1937 (9) that a 1:50,000 solution of adrenalin injected intravenously in man at 2 mgm. per hour had a similar effect. However, the fact that no rise in blood pressure occurs after intracisternal adrenalin and the observation that cerebrospinal fluid withdrawn several hours after intracisternal injection contains adrenalin indicate that adrenalin does not pass from cerebrospinal fluid to blood. Also, adrenalin injected intravenously fails to appear in the cisternal fluid. Thus, adrenalin does not appear to cross the barrier between blood and cerebrospinal fluid in either direction.

Fog (10), Forbes and co-workers (11) have shown that direct application of adrenalin to the pia produces no constriction of the arterioles and only slight constriction of the large arteries; thus the impermeability can not be attributed to vasoconstriction.

Since the blood sugar rise is rapid and high after intracisternal adrenalin, slow and little after intraperitoneal injection and not at all following intra-articular injection and since the blood pressure does not rise after intracisternal injection, intracisternal adrenalin appears to raise blood sugar by acting directly on structures bathed by cerebrospinal fluid.

There is other evidence of the participation of the central nervous system in the effect of adrenalin on the concentration of blood glucose. Aschner (12) showed that hypophysectomized dogs responded to subcutaneous adrenalin with less glycosuria than control dogs. Later De Bodo and his associates (13) found that hypophysectomy minimizes or eliminates the hyperglycemic response to intravenous adrenalin. Winternitz and his co-workers (14) reported in 1944 that in dogs ligation of the carotid sinuses or the vertebral arteries abolishes or minimizes the hyperglycemic response to intravenous adrenalin even when there is adequate liver glycogen. In consequence of these findings, they suggested that the decreased hyperglycemic response to adrenalin following hypophysectomy may be due to injury to neural structures surrounding the hypophysis, particularly those that seem to bear some connection with the carotid sinuses and vertebral arteries.

The analgesic effect of intracarotid and intravenous injection of adrenalin in dogs and of subcutaneous injection in man demonstrated by Ivy and co-workers (15) suggests a direct action of adrenalin upon pain-perceiving centers; so also does Weber's observation (16) that adrenalin injected into the carotid arteries of cats produced analgesia lasting an hour or more. Still more to the point is the finding by Buscaino and Pero (6) that complete analgesia, lasting 1 to 2 hours, could be produced in dogs by subarachnoid injection of 0.1 mgm. of adrenalin.

Finally, central action of adrenalin is indicated by sleep induced by subdural and intracerebral injection of adrenalin in dogs reported by Bass (5), drowsiness in cats and sleep in man observed by us. This effect may explain why spinal anesthesia with procaine is greatly prolonged by addition of small amounts of adrenalin (17, 18).

In connection with the present observations it is of interest to note that Kennard and co-workers (19) found that the blood sugar level in cats is increased for more than 5 hours following frontal lobotomy, but there is no rise in blood pressure.

It seems reasonable to conclude that adrenalin injected intrathecally has several effects due to action on central nervous structures and that among these is the ensuing increase of sugar in the blood. We are still ignorant of the means by which this central action effects the increase of sugar in the blood. From our own experiments we can only conclude that destructions of vagal, spinal including sympathetic, and hypophyseal paths, singly and in pairs were insufficient to prevent or diminish the response.

The present inability to find efferent pathways by way of which the central action of adrenalin causes hyperglycemia is paralleled by unsuccessful attempts to define pathways through which piqûre exerts its effect on blood glucose (20). Since in our experiments the hypophysectomies were not technically perfect, it is still possible that the central hyperglycemic effect of adrenalin was mediated through the remnants of the pituitary. Further work on this point is in progress.

It is conceivable that intracisternal adrenalin exerts its hyperglycemic effect indirectly by way of the circulation: e.g., adrenalin might induce formation of a hyperglycemic factor or might be converted into a hyperglycemic agent which has no pressor properties.

SUMMARY

The barrier between blood and cerebrospinal fluid has been found to be practically impermeable to adrenalin. Adrenalin injected into the subarachnoid space, although it fails to affect blood pressure or electrocardiogram, causes a rapid, high and sustained rise in the concentration of the glucose in blood.

This, like the brief effect on respiration reported here, and the analgesic and soporific effects noted by others, are due to its action on central structures bathed by the cerebrospinal fluid. The site of action and the efferent paths are undiscovered in spite of lesions made to prevent participation of the autonomic nervous system and the hypophysis.

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RELATIVE ACTIVITY OF WRIST MOVING MUSCLES IN STATIC SUPPORT OF THE WRIST JOINT: AN ELECTROMYOGRAPHIC STUDY

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An anatomical investigation in progress (1) dealing with the statics of the wrist joint brings up problems that cannot be solved by strictly anatomical or mechanical methods of analysis. When one holds the wrist horizontally by voluntary muscular action, how do the fifteen muscles traversing the wrist joint share in resisting gravity? Our experience here leads us to believe that an approach to a solution of this type of problem may be made through appropriate action potential experiments.

In maintaining a steady posture by isometric muscular activity, the tension developed in muscles is so adjusted that it nicely balances the load of the hand plus that of objects supported. Torques or moments of force are involved. For static support, i.e., no movement, a torque due to the action of muscles equals the gravity torque. The latter is the product of "weight of the hand (plus objects)" multiplied by the "lever arm" from wrist pivot to the center of gravity of the mass supported. The torque of the antigravity muscles (those muscles above the horizontal level of the wrist pivot) is the product of "muscle tension" times the average "lever arm". Individual lever arms of the various muscles may be represented by the perpendicular distance from the tendons to the center of the wrist pivot (cf. cadaver hands). If one considers the average of the sines of the pertinent muscle lever arms, he may compute a theoretical value for the total effective muscle tension required to balance a given gravity torque. Such a computation, however, gives no information as to individual muscle function. Furthermore, it ignores antagonistic muscular activity and the forces that stabilize against lateral deviation.

Unanswered questions, which have counterparts at other joints as well suggest themselves: Is the theoretical value a fair approximation for the pattern of actual activity? Are all muscles above the wrist pivot tensed to the same degree? Does the muscle with the best leverage tense more or less than others? To what degree are antagonistic muscles active? Do "wrist" and "finger" muscles act differently? Rotation of the forearm about its long axis brings different combinations of muscles above the level of the wrist pivot; are there differences in activity pattern between one muscle combination and another?

The literature on electromyography in relation to human voluntary actions shows close correlations between action potentials and muscle function. Potentials have been studied recently in normal muscles (2–4) and in those affected by disease and denervation (4–6). Either intramuscular needle electrodes or

surface electrodes—or both—have been used. In general, problems of movement—isotonic rather than isometric activity—and the timing of muscle participation in gross activity patterns (7–9) have received most attention. Quantitative correlations between potentials and muscle tensions in isometric activity, however, have not been entirely ignored (3, 10, 11).

We have limited our attention here to a study of the static or isometric aspects of muscle function and have attempted to treat both potentials and torques in a quantitative way. We have worked with ten regions of the forearm muscle mass using surface electrodes over specific muscles. Muscles were carefully identified, and their motor points were stimulated and located as exactly as possible. The electrodes were placed over the muscle with the proximal electrode at the motor point. This procedure allows a fairly precise localization of leads and we believe it gives an approach to the activity of the individual muscles. Our data on muscle action potentials do not seem to be unduly confounded by extraneous action currents.

This report deals with two experiments. The first correlates action potentials with muscle tensions as different known magnitudes of gravity torque are resisted. The second is a determination of the share of specific regions of the muscle mass in the voluntary act of holding a steady posture against a given gravitational force acting from one direction or another.

Procedure. Action potentials obtained from the skin surface overlying specified muscles were amplified by an alternating current, condenser-coupled amplifier and recorded with a General Electric oscillograph. The gain of the amplifier was constant in the range of 60 to 200 cycles and decreased 16 per cent at 20 cycles. The output wave form was satisfactory from 20 to 200 cycles.

The electrodes consisted of two 3/16 inch brass rods to which were soldered silver contact points of the same diameter. The contact surfaces were coated *in vacuo* with a one micron thickness of silver chloride. The electrodes were mounted in a bakelite holder and were adjusted to a spread of 5.5 cm.—about the average length of a forearm muscle fasciculus (12).

Ten of the fifteen muscles traversing the wrist joint were studied.[1] Motor points of each of these muscles were determined and marked on the skin prior to each period of recording. The forearm was placed in a wooden cradle that did not permit pronation and supination of the forearm nor elbow movements. The elbow was bent at a right angle and the forearm was held midway between full pronation and full supination. One electrode was placed over the motor point of a specific muscle with the other electrode lying distally over the muscle. The bakelite holder was strapped with adhesive tape and moist AgCl was placed between the electrodes and the skin. The electrodes, then, were sealed to the skin with celloidin. Stronger action currents were obtained in test

[1] Some muscles were not studied because they were covered by overlying muscles and because motor points could not be determined consistently; these were: Flexor pollicis longus, Abductor pollicis longus, Extensor pollicis brevis, Extensor pollicis longus and Extensor indicis proprius.

records when one electrode overlay a motor point than when the electrodes spanned a motor point or when both electrodes were *proximal or distal* to the motor point.

One of us (W.T.D.) served as subject. Sample records that were comparable were made also on six other subjects as a check. The volume of the hand beyond the wrist pivot (425 ccm.) was determined by water displacement; ignoring the density error, we used 425 grams in calculations of torque. The lever arm of the hand, from the wrist pivot to the center of gravity, was estimated at 7.5 cm. (A standard grip posture was used throughout the study; the locus of the center of gravity was determined from cadaver hands fixed in the standard position; the wrist pivot was located by x-rays of the living wrist; comparable surface landmarks were used in determining the lever arm.) From the hand mass and the lever arm, the gravity torque of the hand was computed as 3.188 kgm.cm. Weights and a fitted hand grip with an extended lever arm were designed to provide torques that were multiples of the hand torque. These were expressed as 2×, 4×, 8×, 16×, 32×, and 64× the hand torque. The latter two were the largest torques that could be lifted by the extensor and flexor muscles respectively.

The axis of the forearm and hand was held horizontal as weights were supported and records were made. In the first experiment, the magnitude of torque supported by muscular action served as the imposed variable. This factor varied from zero, when the hand lay on a supporting surface, through 1×, 2×, and so on to 32× or 64×. Records were made from regions overlying each of the ten muscles studied; the forearm and cradle were rotated for each muscle till the muscle and its tendon were functionally oriented directly above the wrist pivot. In this position, motor point stimulation causes a direct vertical lifting of the hand.

In the second experiment, records were made of each of the ten muscles and a 16× torque was used as a standard load. The forearm and cradle were rotated into twelve positions 30° apart; in this way, the experimental variable was the differing direction of action of the gravity force in relation to the direction of direct muscle pull. Actually a pulley mechanism was used to reverse the direction of the gravity force in those positions which otherwise caused strain and contorted body postures. Comfortable body postures, short periods of weight holding (5–6 sec.) and short rest intervals were used to obviate fatigue effects.

RESULTS. a. *Response to various loads.* Figure 1 shows a representative record from the first experiment; it refers to the Extensor carpi ulnaris muscle region. At *A*, the weight of the hand rests on a supporting surface and the muscle supports no load; at *B*, the hand torque is supported by muscle tension; at *C–G*, multiples of the hand torque from 2× to 32× are supported by muscular action. Patently, the records show an increased amplitude of potential with each increment in torque. A decrease in frequency is less directly apparent. This showed in all the records. The magnitude of potentials, however, varied somewhat from muscle to muscle. The magnitude differed also from

person to person in the supplementary data on six individuals; potentials were less marked in the persons of stronger build.

Our frequency data fall in the range of 35 to 65 per second. For the 1× load, frequencies lie between 50 and 65, and as the load increases to 32×, or 64×, the frequency falls gradually by ten to fifteen counts. The lower frequencies associated with the larger loads are correlated also with higher amplitudes of potential. The various muscles showed no consistent order as to

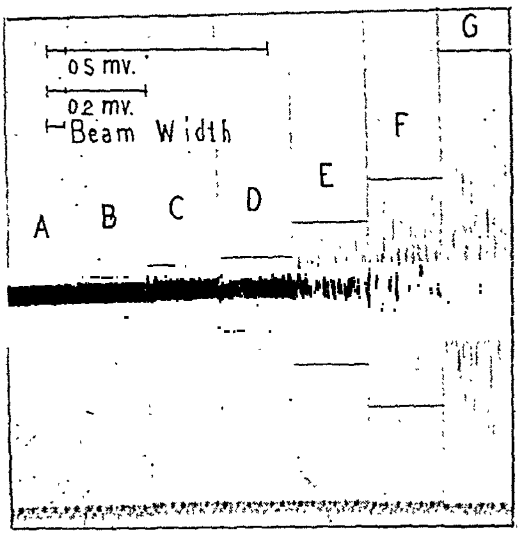

Fig. 1

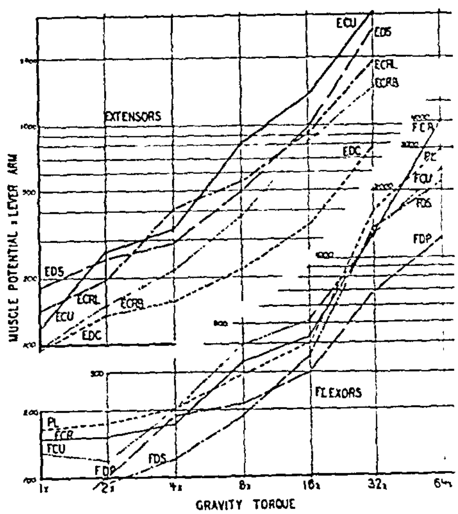

Fig. 2

Fig. 1. Strips from a representative record of muscle potentials (electrodes over Extensor carpi ulnaris muscle) showing increased amplitude correlated with higher degrees of muscle tension. The forearm was oriented so that the muscle lifted weights directly upward. Distance between horizontal lines is the measure of amplitude. *A* resting potential; *B* potential elicited in supporting a 1x torque (the empty hand); *C* record for a 2x torque; *D-G* records involving gravity torques of 4x, 8x, 16x and 32x.

Fig. 2. Muscle potentials (x lever arms) are plotted against loading. Each curve refers to a different forearm muscle below the electrodes. Each muscle supported loads in the direction of its greatest mechanical advantage. Labels are the initial letters of the BNA names for the muscles. If hand and muscle lever arms are assumed to have unit values, the same shaped curve for each muscle represents the relation: potential to weight lifted.

frequency for the various loads, though the five extensors typically (in 88 per cent of the data) showed higher frequencies for a given load than the five flexor muscles. The extensor muscles also showed higher potentials, for a given amount of loading, than the flexor muscles did.

Since frequency changes are not as striking in the records as amplitude, the latter factor will henceforth receive prime attention. Our measure of potential is shown in figure 1 as the distance between the horizontal lines on each of the record strips. It was determined by a statistical treatment of measurements of the distances between the successive peaks and a baseline; these data were used directly and in practice the voltage calibration shown in the figure was

not used. Our potential value was the sum of the following factors: *a*, the difference between the average of the upper peaks and the average of the lower peaks (minus the beam width), plus *b*, 3 standard deviations (sigma) of the upper peaks, plus *c*, 3 sigmas for the lower peaks. This measure of range should include 99.7 per cent of all the peaks for this type of data.

Frequency and voltage are used here in a purely empirical way to bring out differences in the electrical record which correlate with varying degrees of loading of the hand. No attempt is made to interpret physiologically the apparent piling up of the oscillographic tracing for the heavier loads.

In figure 2, potentials (times the lever arms of the pertinent muscles) are plotted on log-log co-ordinates against the torques that are supported by muscular action. (The muscle lever arms, here, are mere proportionality factors which vertically raise or lower the curves relative to one another without changing their individual form; their use, however, harmonizes with a basic treatment consistent with torques.) If the gravity and muscle lever arms are regarded for the moment as unit values, each curve suggests the relation between muscle potential and the weights supported. Though the potential values for a given load differ from muscle to muscle, the curves are in general similar; that is, in the log-log plotting used, the curves have a somewhat similar contour and they are more or less parallel to one another. They show a definite correlation between increments of potential and increments of torque though it is not quite a straight line relationship. Averaged curves for the flexors and for the extensors show a smooth curved contour slightly concave to the left. The averaged extensor curve is slightly straighter than that for the flexors. For each muscle, potential increases with each increment in the gravity torque supported (cf. fig. 1).

Extensor muscles show consistently higher potentials for a given load than flexors. In general, extensors resisting a 32× torque produce potentials as large as flexors resisting a 64× torque. Except for the lowest torques (1× and 2×), extensor potentials are matched somewhat closely by flexor potentials produced at twice the extensor torque values. This observation is correlated with the facts that the flexor mass is roughly twice as great as the extensor mass and that maximum flexor strength is about twice the extensor strength also.

Since, as stated in our introductory paragraphs, the gravity torque is balanced exactly by a torque due to tension in the muscle mass acting over its lever arms, it follows that the abscissa in figure 2 can be visualized in terms of relative muscle torque as well as for gravity torque. In this sense each curve represents a correlation between the potentials recorded from regions over specific muscles (times the muscle lever arm) and the muscle torques which just support specific amounts of loading. This correlation will have significance later, since it will permit a calibration of experimentally varied potentials from the region of specific muscles in terms of either torques or relative tensions.

b. *Response to differing directions of action of a gravity torque.* When the forearm and supporting cradle are rotated through twelve stations, 30° apart,

the standard 16× torque is directly supported by one combination, then another, of the wrist supporting muscles. In the first position, a selected muscle is a direct supporter; in the second position its direction of pull becomes oblique to the axis of direct support; in subsequent positions its action is directed laterally, then downward and finally it acts through angles of 180° to 360° till it again approaches a position of direct support. Records were made at each of the stations; in this way action potentials that were correlated with agonist, antagonist, oblique and lateral pulls of the muscle mass were obtained.

Figure 3 shows these data plotted on polar co-ordinates for a representative wrist muscle. Shading differentiates the agonist (larger area) and antagonist (smaller area) quadrants from the white lateral stabilizing quadrants. In the direction of the arrow the muscle pulls the 16× load directly against gravity; the potential in this direction is maximum. When other muscles take over the direct supporting function and this muscle pulls obliquely the potentials are definitely less. There is a further decrease in potentials as the direction of pull becomes horizontal, that is, at a right angle to the downward direction of gravity, potentials in the antagonist quadrant are about a third of the maximum potential for supporting the load.

Since the only experimental variable here is the changing direction of muscle pull in relation to the direction of action of the gravitational force, the variations in potential in such a graph must indicate differing degrees of muscular activity. A correlation between potentials recorded over specific muscles and the gravity torques resisted by muscle action has been shown. Furthermore it has been indicated that the potentials are correlated also with the muscle torque due to the muscle mass which is mechanically so placed as to resist gravity. A calibration of data such as those of the polar graph may be made directly from the curves of figure 2 or smoothed average curves for the extensor muscles and for the flexors may be used. Results by either method permit the same conclusions but the latter method was arbitrarily adopted here.

The smoothed curves, on transparent film, were adjusted over a large log-log graph with the 16× position on a curve superimposed on the potential value that was obtained for the direction of direct muscle pull in the second experiment. The equivalent torque values were then read off the graph for each of the potentials obtained in the various angular positions. Figure 3 is not greatly changed in form when equivalent torques are substituted for potentials.

Figure 4 shows data from regions overlying six other muscles treated in this way. All of the graphs have the same orientation in relation to the wrist, the three upper graphs with arrows indicating the direction of direct muscle pull deal with extensor muscles, the three lower figures refer to flexors and the arrows are directed oppositely. The concentric circles or parts of circles represent a calibration in terms of torque: 16×, outer; 8×, middle; and 4×, inner. The agonist, antagonist, and lateral stabilizing quadrants are outlined by the white lines.

The most common pattern encountered in our data is shown by the Extensor-digiti-5 graph at the upper left. Torque is maximum in the direction of direct

muscle pull. It decreases in each direction through the agonist and lateral stabilizing quadrants. In the lateral stabilizing position (90° from the mid-agonist direction) the torque value is about half maximum. In the direct antagonist position activity is about one-fourth maximum. Data not shown here but relating to the Palmaris longus, Extensor carpi ulnaris and Extensor carpi radialis brevis muscles show this pattern also. Though the other graphs shown in figure 4 deviate in certain ways, the type pattern is shown also on the left side of the upper middle figure, on the right side of the upper right figure and on the right side of the middle lower figure. In the ten muscles studied, twelve lateral halves of the graphs show a similar pattern; we regard these as typical. The other eight halves are all shown in figure 4. These deviations in pattern, it is believed, can be accounted for, as described below.

It should be understood that we are without information as to what part of the muscle mass below the electrodes is recorded in our data. It may be assumed as a first approximation that the muscle directly below the electrodes contributes significantly to the electrical record. The data, then, may be examined critically for corroborating or contradictory evidence.

The axis of the egg-shaped form of the more typical polar graphs (fig. 4, upper left; cf. fig. 3) slants in the direction of direct muscle pull for the muscle that lies below the electrodes. In the other graphs of figure 4, the maximum torque has an angular spread on each side of the direction of pull by the muscle under consideration. An axis, however, through the middle of the agonist and antagonist quadrants, that is, along the direction of pull, roughly divides the graph into symmetrical halves. These observations are compatible with the assumption that the muscle below the electrodes affects the graph very significantly.

In figure 4, the graph for the Extensor digitorum communis muscle (upper middle) shows a spur toward the right that is greater than the 16× torque; this supra-maximal torque must be regarded as an artifact due to adventitious electrical influences from the adjacent Extensor carpi radialis muscles. These

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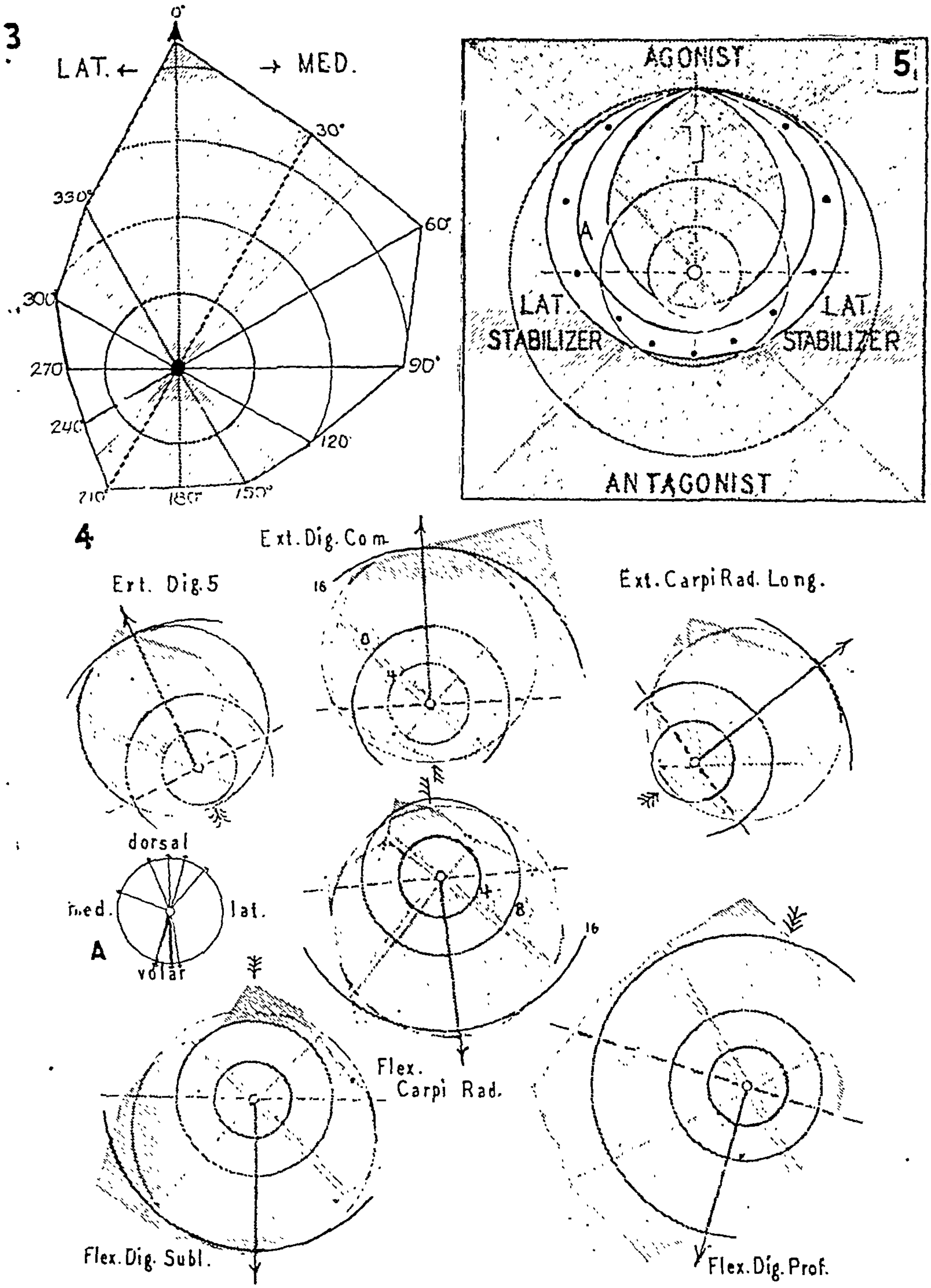
Fig. 3. A polar co-ordinate graph showing the relative magnitudes of potentials (Palmaris longus muscle region; 16x torque supported) as the forearm is rotated through 360°. The direction of greatest mechanical advantage is upward, 0°; the larger gray area represents the agonist quadrant. The smaller area is the region of antagonist potential vectors, White (lateral stabilizing action) quadrants show decreasing potentials as muscle action changes from agonist to antagonist.

Fig. 4. Derivative curves of muscle torque showing the degree of activity in various directions of muscle pull in relation to the magnitude in the direction of best mechanical advantage (the arrows). Data on six muscles are shown. The arrows in sketch A show the orientation of direct muscle pull for ten muscles including these six. Agonist, antagonist and lateral stabilizing quadrants are separated by white lines.

Fig. 5. A generalized curve (*A*) showing the relative activity of a typical forearm muscle (?) in the different quadrants as the forearm was rotated through 360° and a given load was supported. The arrow represents the direction of straight muscle pull. The three concentric circles (from large to small) represent maximum, half maximum and quarter maximum torque values. The white annulus includes torque vectors falling within $\pm$ 1σ of the average curve *A*. Curve *A* is an average based on selected "typical" muscles.


3
0°
LAT. ←
→ MED.
30°
330°
60°
300
270
90°
240
120
210
180
150°
5
AGONIST
A
LAT.
STABILIZER
LAT.
STABILIZER
ANTAGONIST
4
Ext. Dig. Com.
Ext. Dig. 5
16
8
4
Ext. Carpi Rad. Long.
dorsal
med.
lat.
A
volar
4
8
16
Flex.
Carpi Rad.
Flex. Dig. Subl.
Flex. Dig. Prof.

Figs. 3-5

latter muscles, in all our records responded with a higher potential to a given torque than the other extensors. A graph of the Flexor carpi ulnaris muscle (not shown) is distorted in certain directions by the clearly recognizable influence of the immediately underlying Flexor digitorum profundus muscle. Supramaximal spurs and asymmetries can probably be accounted for in this manner (cf. Flex. dig. subl). These effects are on the whole localized or minor. Certain asymmetries may be due to random influences (Flex. carpi rad.) and if recordings had been made at finer intervals than 30° they could be better understood.

The Flexor digitorum profundus (lower right graph) is unique and is of special interest. Electrodes were placed over the region of the muscle that flexed the fourth and fifth fingers. In the direction of the arrow, the muscle acted as a wrist supporter; when the forearm was rotated 180°, the 16× load bore on the fingers. The high torque in this direction and in the whole arc to the left (ulnar side) was due to strong flexion of fingers 4 and 5, while the muscle was otherwise a wrist antagonist or stabilizer.

The greater than usual antagonist torque of the Flexor digitorum sublimus is doubtless due also to finger flexion when the muscle was otherwise an antagonist; in this instance the electrodes overlay the muscle slip to the median finger —the proximal finger joints were not called into as vigorous action as the series of joints that are moved by the Flexor digitorum profundus.

From a check on the deviating patterns in relation to the more typical, we feel that the electrical record relates primarily to the muscle below the electrodes, but that there are slight influences due to the electrical state of adjacent muscles. The deviations of the finger flexors are additional evidences that the electrical record is primarily due to a specific muscle.

Discussion. The electrical activity that is recorded in our electromyograms from regions of the forearm clearly correlates with muscle tensions. As larger loads are supported, muscle tensions must be increased proportionately; leads over individual muscles transmit currents that can be correlated with these factors. Our method of tapping one region, then another, of the forearm muscle mass does not justify direct comparison of potentials from the various regions in terms of muscle forces since local conditions vary (i.e., fascial padding, possible electrical variations between muscles, etc.). Methods of simultaneous recording, if equipment had been available, might have given data suitable for this. We believe that there is reasonable evidence, apart from minor adventitious influences from adjacent muscles, to assume that the electrical records relate primarily to the muscle adjacent to the electrodes. Multiple recordings and methods using needle electrodes would be a desirable supplement.

By equating the potentials recorded from specific regions with the gravity torques (= muscle torques) due to loads supported, it is then possible to compare the activity of one muscle region with another. A number of the forearm muscles show a pattern that has common characteristics: agonist activity in the direction of straight muscle pull is maximum; strength decreases as the agonist function changes toward lateral stabilization; muscle action then further

decreases to a fraction of maximum as the muscle assumes an antagonist rôle. When the patterns are oriented so that the direction of muscle pull coincides in all, an average pattern of activity emerges (fig. 5). Line *A* in the figure represents the average pattern when peculiarities in certain records due to finger action and adventitious effects (discussed above) were discarded; right and left sides were summated and the curve was drawn symmetrically. The white on each side of *A* represents the standard deviation. When the suspect data are included, the circle of dots represents the average pattern.

The agonist torque, which in our data has a maximum value when a load 16× the hand torque is supported, decreases gradually to about 8× as muscle function changes from agonist to lateral stabilizer; the torque further decreases to 4× (or 5×) when the muscle acts as an antagonist.

Figure 5 relating to the activity of typical muscles in the voluntary act of supporting loads, with the wrist held in a steady posture, may be interpreted thus: a muscle, in a position of maximum mechanical advantage, contracts more strongly than it would if it acts obliquely to the disturbing force. Furthermore, it is apparent that lower but appreciable joint stabilizing forces are exerted by muscles when they are not directly supporting loads. This activity, but to a relatively low degree, however, is manifest in the antagonist quadrant. Possibly at joints where ligaments (collaterals) bear the brunt of stabilizing activity, such stabilizing effects of muscles might be negligible. Load supporting muscles must simultaneously support the load and counteract the forces of antagonists.

The implications of this type of study are broader than the specific emphasis discussed. If the typical wrist moving muscles respond in patterns such as outlined here, the control of a whole group of such muscles must be modulated by stereotyped reflex mechanisms, possibly at cord levels. It follows, then, that proprioceptive stimuli from these muscles in varying degrees of isometric contraction, must be summated in patterns that are equivalent to the tension patterns which exist. Stimuli should vary as one combination of muscles or another takes over the agonist function but a pattern of a set type should persist. Some kind of stereotyped association pattern likewise must be assumed to exist in neural centers and to mediate patterns of stimuli and patterns of response that are involved in this simple type of isometric reflex.

SUMMARY

1. Electromyograms from each of ten regions of the forearm muscle mass were made with surface electrodes while the hand, held stable and horizontal, supported known loads.

2. Potentials from each of the ten regions reflected more or less closely the activity of specific forearm muscles underlying the electrodes.

3. A statistical measure of range applied to records of potentials showed increasing values with increased loading of the hand; the latter factor was expressed as multiples of the hand torque.

4. Since loads were counterpoised by a summation of muscle tensions, poten-

tials over a specific muscle reflected more or less the participation of this muscle in the total tension pattern.

5. When the forearm and hand were rotated so that gravity acted at different angles to the plane of action of a given test muscle, potentials from the region overlying the muscle varied with the angular position.

6. Typically, potentials were maximal when a muscle underlying the electrodes was in the direct agonist position; then the activity of the muscle was expended in directly counteracting gravity.

7. The tension of a muscle (as reflected in potentials) gradually decreased to about half maximum as the agonist function gave way to lateral stabilizing; in this position the test muscle acted in the same horizontal plane as the wrist pivot.

8. With further hand and forearm rotation, the test muscle became an antagonist, working with gravity to stabilize the carpus, and activity, as measured by potentials, was a third to a quarter of the maximum agonist value for a given loading.

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AN EXPERIMENTAL STUDY OF EXPLOSIVE DECOMPRESSION INJURY[1]

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Explosive decompression may be defined as a sudden exposure to lowered environmental pressure. In common usage the term has come to connote exposure to rapidly lowered barometric pressure, as encountered by aircrews when failure of cabin pressurizing equipment occurs (due to mechanical failure in the aircraft, damage from gunfire, etc.), or in emergency conditions necessitating the leaving of aircraft at high altitude. Hence, studies on the effects of suddenly lowered environmental pressure are usually viewed as constituting a part of the literature of aviation physiology. However, similar conditions prevail in deep-sea diving (accidental "blow-up" from great depths) and, indeed, in any too-sudden ascent, as in submarine "free-escape" and similar situations. The subject of explosive decompression injury thus applies to submarine physiology as well as to the specialty of aviation medicine.

METHODS. In the experiments to be described, 10 cats and 372 rats were subjected to 495 explosive decompressions. Cats were decompressed to simulated altitudes up to 35,000 ft. in 1.5 sec., while rats were similarly exposed to pressure equivalents of 50,000 ft. in 1.06 sec. and to 80,000 ft. in 0.06 sec. The time of decompression was calculated from high-speed motion pictures of the manometers connected with the various chambers used.

Two methods of recompression were used, arbitrarily characterized as "immediate" (within 10 sec., maximum time) and "slow". In the "slow" method, recompression to the 18,000-ft. level was carried out at an average rate of 200 m.p.h., and thereafter at 20 f.p.s. This was done in an attempt to simulate the effects of free-fall with subsequent parachute descent on attaining the 18,000-ft. level. Since, under present operating conditions, recompression would take place at approximately the latter rate, it was felt that these experiments were of a more practical nature than were the former—"immediate" recompression being now possible only under laboratory conditions. E. C. G. and respiratory tracings were secured by means of a Grass electro-encephalograph, suitably filtered. Blood pressure was read from a mercury manometer directly connected to the cannulated carotid artery.

Pathology. Hemorrhage into the pulmonary tissues was the single constant finding, as pointed out by Latner (7). The damage was, in all cases observed, confined to those structures containing gas. In this respect the pathology was strikingly reminiscent of that resulting from exposure to blast, as described by Greaves *et al.* (4). Its similarity to the reported pathology resulting from "blow-

[1] The experiments described were undertaken as part of a program of research performed under contract (N6-ori 116) with the United States Navy, Office of Naval Research.

up" in divers as well as from accidents occurring in "free-escape" (in instances in which the breath was held during ascent) was, moreover, notable. This similarity in pathology would be expected as in keeping with Boyle's law of

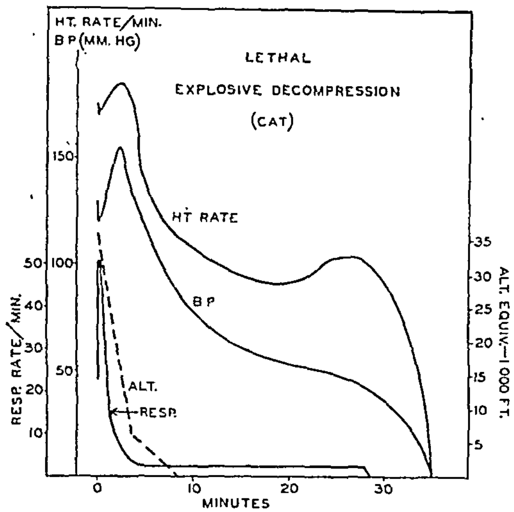

Fig. 1

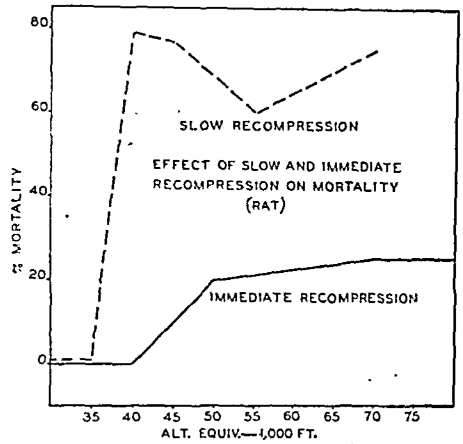

Fig. 3

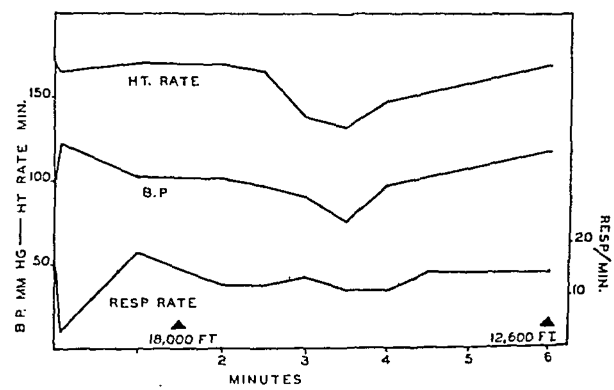

Fig. 2

Fig. 1. Lethal explosive decompression in the cat. The animal was recompressed at a rate of 200 m.p.h. until the 18,000-ft. level was attained. Thereafter "descent" was limited to 20 f.p.s. The effects on heart rate, blood pressure and respiratory rate are shown.

Fig. 2. Non-lethal explosive decompression in the cat. The curves represent an average for 6 animals recompressed by the "slow" method as in figure 1. The transitory effects upon heart rate, blood pressure and respiratory rate are shown.

Fig. 3. The effect of "slow" (172 rats) and "immediate" (200 rats) recompression on the mortality rate of animals explosively decompressed to simulated altitudes up to 80,000 ft.

expanding gases, since at an altitude of 55,000 ft., for example, a gas will expand to approximately 11 times its volume at sea level, and only the "valving" of gas through the respiratory tree can account for survival in instances of explosive decompression to such altitudes.

Explosive decompression injury was found to be characterized by diffuse

intrapulmonary hemorrhage which varied, in individual instances, from minute, petechial lesions to gross hemorrhage involving all of the pulmonary tissues. No lesions were observed in animals decompressed to simulated altitudes below 35,000 ft. In the present series, the right lung showed more extensive trauma than did the left in 55 per cent of cases, while in both lungs the apical poles, posterior surfaces and thin, pulmonary margins appeared to be the most vulnerable areas. Perforation of the gut never occurred in the cats studied, and in but 3 of the 372 rats. There was no correlation between the weight of the animal decompressed and the extent or degree of pathology exhibited. That is to say, proportional injury was found in both large and small animals exposed to the same degree of decompression.

Circulation and respiration. Immediately following decompression, both the blood pressure and heart rate decreased sharply. This, together with the engorged hearts found at autopsy, was ascribed to mechanical interference with cardiac output, and it is suggested that pressure upon the heart may result from the momentarily increased intrathoracic pressure resulting from supra-maximal expansion of the lungs—an opinion held by Whitehorn *et al.* (9). In fatal cases, both heart rate and blood pressure were observed to fall progressively until death (fig. 1), while, in a few instances, marked, alternate accelerations and decelerations in heart rate were seen during the few minutes prior to complete stasis.

The E. C. G. of fatal explosive decompression injury was found to be simply that of a dying animal, and the characteristics of the tracings obtained were not considered as being in any way unique. Arrhythmia was a constant finding in the moribund heart, with extra systoles, decreased "R"-spike potentials and abnormalities of the "T" wave. Animals which survived (fig. 2) frequently showed no change in heart rate or bioelectric complex, but initial slowing of the heart was the rule. After animals which survived "slow" recompression had been brought to about 15,000 ft. simulated altitude (2.5 min.), a fall in heart rate and blood pressure usually occurred from which recovery to normal values was complete before recompression had reached the 10,000-ft. level. Respiratory rate and depth in such animals was little affected, except during the first minute of the descent, when respiratory action was slow and dyspneic.

"Group" (simultaneous) decompression. In experiments in which several rats were simultaneously decompressed, the pathology was found to be particularly interesting (table 1). In these animals, decompressed to 80 mm. Hg (about 50,000 ft.) and recompressed at the "slow" rate, mortality was found to approximate 50 per cent, although all animals had been exposed to the same degree of decompression for the same length of time. Furthermore, on autopsy, highly variable degrees of pulmonary injury were seen, and the appearance of the lungs of the different animals might vary from the normal to gross, generalized hemorrhage involving almost all of the pulmonary tissues. Intermediate degrees of injury could also be seen in these instances of "group" decompression, varying from a few isolated, punctate, hemorrhagic spots to diffuse areas covering large portions of the lung.

It was considered most probable that this highly-variable pulmonary damage might best be explained on the basis of varying phases of the respiratory act at the instant of decompression, since all gas present within the lungs would expand in equal proportion and be "valved" through the trachea at rates corresponding with the size of the individual animals. This hypothesis appeared more tenable as an explanation of the observed variability in degree and extent of injury than considerations of possible anatomical differences in the respiratory tree which might permit the "valving" of the expanding gases at different rates and pressures.

Protection. As a partial test of the above hypothesis, a series of animals was decompressed following the tight mechanical constriction of both thorax and abdomen. Such constriction was accomplished by means of surgical tape so applied as to permit but minimal pulmonary exchange. All of these rats survived, and on autopsy injury was found to be, indeed, minimal, although hemorrhagic areas were present in all of the lungs examined (table 2):

TABLE 1

Example of pathological variation in simultaneous ("group") explosive decompression of rats to a simulated altitude of 50,000 ft.

A. Died within 5 minutes. Massive hemorrhage involving almost entire surface of both lungs
B. Died within 15 minutes. Gross hemorrhage into both lungs
C. Survived. Gross hemorrhage along borders of both lungs
D. Survived. Diffuse hemorrhage involving the apices of both lungs. The remainder of the pulmonary tissues appeared normal
E. Survived. Both lungs normal in appearance

Rate of recompression as a factor in survival. Experiments involving 372 decompressions indicated that the lethal effects of explosive decompression are dependent, to some degree at least, upon the duration of the subsequent period during which the animal was restored to normal atmospheric pressure.

Thus, in rats decompressed to a simulated altitude of 80,000 ft. (20–35 mm. Hg) and immediately recompressed, mortality never exceeded 25 per cent; while rats more slowly brought to normal barometric pressure suffered mortality rates up to 78 per cent (fig. 3). Moreover, in rats recompressed by the "slow" method, fatalities occurred at simulated altitudes as low as 35,000 ft., while no deaths took place among rats "immediately" recompressed until the 40,000-ft. level. No explanation for this difference in survival percentages is offered, but the possibility is suggested that rapid recompression may tend to minimize hemorrhage by the speedy reduction of tension on the finer pulmonary capillaries.

Discussion. The pathology of explosive decompression is now well known in its essentials (1, 2, 5–9). Differences of opinion exist, however, as to the immediate cause of death, whether from the formation of gas emboli (6), anoxic anoxia (3), anemic anoxia and shock, or from pulmonary trauma. There is

general agreement, on the other hand, that structural damage, when it occurs, appears like blast injury to be limited to those structures containing gas. In addition, Livingston *et al.* (8) have described renal and cochlear lesions together with a generalized passive congestion in animals examined within a few days following decompression.

The changes in respiratory rate, heart rate, blood pressure, and in the cardiac bioelectric complex are most probably initiated by mechanical changes within the thorax. Whether or not these changes are of a transitory nature appears

TABLE 2

Post-mortem findings in rats "protected" against explosive decompression by means of tightly taping the thorax and abdomen in such a manner that only minimal pulmonary ventilation was permitted. All animals survived and were sacrificed 8 hrs. following the decompression

NO.	SIMULATED ALTITUDE	PATHOLOGICAL FINDINGS
1	50,000 ft.	Lungs normal with the exception of hemorrhagic streaks on the posterior border of the right lung
2	50,000 ft.	Both lungs mildly hemorrhagic and pink in color. The right lung appeared somewhat darker in color than the left. Single, grossly hemorrhagic spot on right lung
3	50,000 ft.	Similar to no. 45 above, with single grossly hemorrhagic area in left lung
4	50,000 ft.	Both lungs presented numerous, small and discrete hemorrhagic lesions
5	51,000 ft.	A few small petechial spots on both lungs, with a single grossly hemorrhagic lesion on the right side

to depend upon the degree of trauma brought about by the initial insult. Thus, Whitehorn *et al.*, working with dogs, found explosive decompression to be harmless in all but one instance in which decompression was effected from 10,000 to 50,000 ft. simulated altitude. It should be noted that recompression, in these experiments, was immediate. Although the mortality figures derived from the present experiments with rats are not in complete agreement with the results of the experiments on dogs cited above (but 75 per cent of rats decompressed to 80,000 ft. surviving) it appears, nevertheless, that the rate of recompression is definitely a factor in determining survival.

Thus, in experiments in which animals were recompressed at such a rate as to simulate free-fall from aircraft and parachute descent, only 22 per cent survived, as compared with the above 75 per cent survival for rats "immediately" recompressed. It thus appears desirable that experiments performed for the purpose of evaluating the hazard of explosive decompression in man be designed so as to simulate, as closely as possible, conditions prevailing in failure of pressurizing equipment or in emergency conditions necessitating parachute descent from aircraft. Particularly is this so since to date "immediate" recompression of explosively decompressed personnel is (and will probably remain for some time) impractical, if not impossible of achievement.

SUMMARY

1. Cats and rats were subjected to 495 explosive decompressions to simulated altitudes up to 80,000 ft.

2. Explosive decompression injury was found to be confined to those structures containing gas. In the lungs, damage may vary from petechial lesions to gross hemorrhage involving all of the pulmonary tissues.

3. No correlation was found between the weight of the animals decompressed and the extent of resultant injury.

4. Fatalities from explosive decompression did not occur at simulated altitudes below 35,000 ft.

5. In fatal explosive decompression, both heart rate and blood pressure declined rapidly until death. No unique criteria were detected in the E. C. G. of such animals—those phenomena observed being characteristic of any dying heart. Animals which survived exhibited a fall in both heart rate and blood pressure from which recovery was complete before recompression had reached the 10,000-ft. level.

6. In "group" decompressions, marked variations in pathology were observed. These may be tentatively explained on the basis of varying phases of respiration at the instant of decompression. The "protection" of rats by means of tightly taping the thorax and abdomen was interpreted as supporting this hypothesis.

7. The rate of recompression appeared to constitute a factor determining the degree of damage sustained as well as in affecting the mortality rate of rats explosively decompressed.

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THE EFFECT OF ALLOXAN ON MUSCLE GLYCOLYSIS[1,2]

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There have been several references to the action of alloxan on enzyme inhibition. Purr (1) has shown that alloxan inhibits the activity of papain and cathepsin, and Hopkins, Morgan and Lutwak-Mann (2) have demonstrated the same effect on the succinic dehydrogenase. Also, alloxan can act as a hydrogen acceptor in enzyme reactions (3, 4) and to compete with diphosphopyridine nucleotide in yeast fermentation (5). Alloxan has been shown to enter a reversible oxidation-reduction system (6) which is unstable above a pH of 6.0. Since alloxan can act as an oxidizing agent and inhibit enzyme activity, it was decided to investigate the action of alloxan on the glycolysis of glycogen to lactate in muscle extracts.

METHODS. The method used was essentially similar to that of Gemmill and Hellerman (7). The muscles of the hind legs of frogs were quickly dissected and 1.5 parts of cold water were added to each part of muscle. The mixture was ground with sand in a chilled mortar and centrifuged. The supernatant fluid was used for the determinations. In each Warburg vessel was placed 0.4 cc. of 1.3 per cent sodium bicarbonate, 1.0 cc. of the extract, 0.2 cc. of alloxan of a strength which varied with the different experiments and 0.2 cc. of 4.0 per cent glycogen was placed in the side tube. Water was added to make the final volume in each vessel 2.2 cc. In the earlier experiments the alloxan was added to the bicarbonate mixture before the muscle extract. In the later experiments, the alloxan was added after the muscle extract was placed in the vessel in order that the alloxan would not be subjected to the alkaline solution. After placing the vessels in the water bath, 95 per cent nitrogen and 5 per cent carbon dioxide were passed through the vessels for six to eight minutes. At the end of this time the passage of gas was discontinued and the side tubes were closed. The vessels were shaken for another five minutes before the first reading was taken, the stopcocks were closed and the glycogen was spilled over into the main chambers of the vessels. Readings were taken for a period of one hour. The temperature of the water bath was 25.0°C. In a few experiments lactate determinations were made by the method described by Edwards (8).

RESULTS. The results are given in the figures 1 and 2 and in tables 1 to 3. In the early experiments in which the alloxan was added to the bicarbonate solution before the muscle extract, it was found that relatively large amounts of alloxan were needed in order to get 88 per cent inhibition (table 1). In later experiments

[1] These experiments were aided in part by a grant from the Eli Lilly Research Fund.

[2] Experiments reported before the Am. Soc. Pharmacol. and Exper. Therap., Fed. Proc. **6:** 332, 1947.

TABLE 1

The effects of alloxan on glycolysis

Experiments made with 1.0 ml. extract (1 part muscle, 1.5 parts water), 0.2 ml. of 4 per cent glycogen and 0.4 ml. of 1.3 per cent sodium bicarbonate. Alloxan solution and water added to make total volume in each vessel 2.2 ml. Solutions saturated with 95 per cent nitrogen and 5 per cent carbon dioxide in water bath at 25.0°C.

Alloxan added before extract

DATE	ALLOXAN	GLYCOLYSIS		INHIBITION
		Without alloxan	With alloxan	
1947	*mgm.*	*mm.³ CO_2*	*mm.³ CO_2*	*per cent*
Jan. 27	1.2	358	338	6
		364	335	8
		371	338	9
28	2.4	469	103	88
		491	102	89
		490	104	89
31	3.6	337	116	66
		358	80	88
		351	174	50
Feb. 1	4.8	400	187	53
		405	107	74
		409	133	67

Alloxan added after extract

DATE	ALLOXAN	GLYCOLYSIS		INHIBITION
		Without alloxan	With alloxan	
1947	*mgm.*			*per cent*
Feb. 3	3.6	386	49	87
		395	51	87
		405	53	87
5	3.6	286	21	93
			25	91
6	3.6	326	47	86
			49	85
7	2.4	399	30	93
			24	94
8	0.2	410	410	0
	0.4	415	221	47
	0.6		77	82
	0.8		43	90
10	0.8	263	26	90
			16	94
17	0.8	278	26	91
			28	90

TABLE 2

Cysteine reactivation of alloxan inhibition of glycolysis

Experiments made with 1.0 ml. extract (1 part muscle, 1.5 parts water), 0.2 ml. of 4 per cent glycogen and 0.4 ml. of 1.3 per cent sodium bicarbonate. Alloxan solution and water added to make total volume in each vessel 2.2 ml. Cysteine hydrochloride neutralized with sodium bicarbonate added ten minutes after alloxan. First reading was made twenty minutes after the addition of the cysteine to the solutions. Solutions saturated with 95 per cent nitrogen and 5 per cent carbon dioxide in water bath at 25.0°C. N, without alloxan; NC, without alloxan, with cysteine; A, with alloxan; AC, with alloxan and cysteine.

DATE	ALLOXAN	CYSTEINE HCl	GLYCOLYSIS, MM. CO_2 PER HOUR			
			N	NC	A	AC
	mgm.	*mgm.*				
Feb. 6	3.6	4	326	530	47	25
					49	27
7	1.2	30	399	382	24	200
					30	220
10	0.8	15	263	336	16	156
					26	156

in which the alloxan was placed in the vessels after the extract, inhibition was obtained with 0.6 mgm. of alloxan (fig. 1 and table 1). There can be no doubt that the stronger alkalinity to which the alloxan was subjected in the earlier experi-

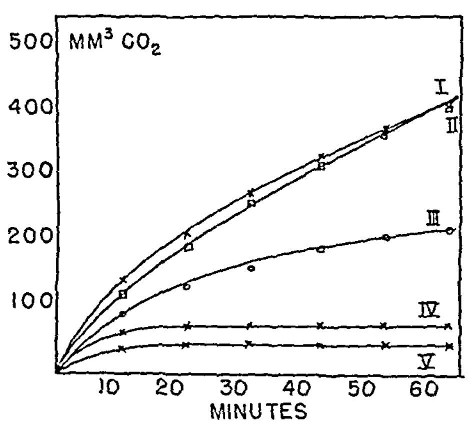
TABLE 3

Effect of alloxan on lactic acid formation in muscle glycolysis

Lactic acid in mgm.

BEGINNING		END OF 1 HOUR	
Without alloxan	With alloxan	Without alloxan	With alloxan
0.62	0.46	1.40	0.44
		1.36	0.44

Solutions used: 1 ml. extract (1 part muscle, 1.5 parts water); sodium bicarbonate, 0.4 ml. of 1.3 per cent; glycogen, 0.2 ml. of 4.0 per cent; alloxan, 0.2 ml. of 0.4 per cent. Water added to each vessel to make final volume 2.2 ml. Solutions equilibrated with 95 per cent nitrogen and 5 per cent carbon dioxide in water bath at 25.0°C .



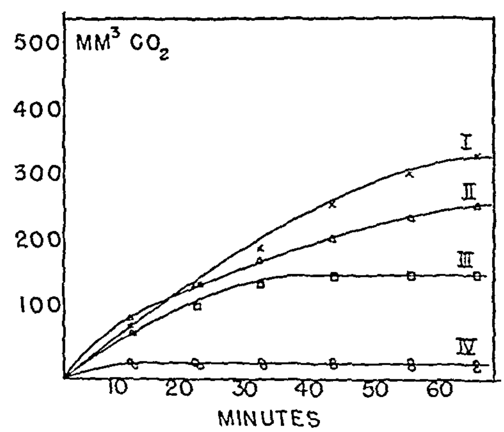


Fig. 1. Effect of varying concentrations of alloxan on glycolysis. Concentration of solutions: glycogen, 0.2 ml. of 4.0 per cent; sodium bicarbonate, 0.4 ml. of 1.3 per cent; muscle extract, 1.0 ml.; *I*, no alloxan; *II*, 0.1 ml. of 0.2 per cent; *III*, 0.2 ml. of 0.2 percent; IV, 0.3 ml. of 0.2 per cent; V, 0.4 ml. of 0.2 per cent. Final volume in each vessel adjusted to 2.2 ml. with water; 95 per cent nitrogen and 5 per cent carbon dioxide; 25.0° C.

Fig. 2. Effect of alloxan and cysteine on glycolysis. *Concentration of solutions*: glycogen, 0.2 ml. of 4.0 per cent; sodium bicarbonate, 0.4 ml. of 1.3 per cent; alloxan, 0.2 ml of 0.4 per cent; cysteine, 0.3 ml. of 5 per cent. Final volume in each vessel adjusted to 2.2 ml. with water; 95 per cent nitrogen and 5 per cent carbon dioxide; 25.0° C.

Curve *I*, cysteine; curve *II*, normal; curve *III*, alloxan and cysteine; curve *IV*, alloxan.

ments was destroying some of the alloxan. The relationship between concentration and the degree of inhibition with this latter method is given in figure 1.

Since alloxan may act as an oxidizing agent, it was decided to determine if cysteine may reactivate this inhibition. Alloxan was added to the muscle extract and the mixture was allowed to stand for ten minutes before the cysteine was added and the experiment started. The results given in figure 2 and table 2

show that the cysteine does relieve this inhibition. It is of interest to note that the reactivation only occurs during the first thirty minutes of the experiment. After this time the curve again becomes parallel to the base line indicating inhibition was taking place again. The cysteine was not exhausted at this point because the solution gave a strong nitroprusside test for sulfhydryl groups at the end of the experiment. In another experiment, cysteine was added fifteen minutes after the alloxan and similar reactivation was obtained.

In a later experiment, determinations of lactic acid were made before and after a manometric experiment (table 3). No increase of lactate was observed when alloxan was present while in the absence of alloxan, lactate was found in the reaction mixtures. These results demonstrate also that alloxan was actually inhibiting the glycolysis of glycogen to lactate.

Several months after completing these experiments, an additional experiment was made using the same sample of alloxan as in the former work. Only a slight inhibitory action was obtained. The next day, using a new sample of alloxan, the same results as in the previous series were obtained. Therefore, care must be observed to use a fresh supply of alloxan for biological experiments.

Discussion. Lipmann (9) has reviewed the reversible effects of oxidizing agents on glycolysis. He remarks that there are five enzyme systems in the glycolytic system which undergo oxidative inactivation followed by reactivation with sulfhydryl compounds. In the present experiments, the exact enzyme system cannot be defined; however, alloxan can be added to the list of oxidizing agents which can reversibly inactivate glycolysis.

The relationship of these experiments to the mechanism of the action of alloxan on the islets of Langerhans is open to conjecture. It would be interesting to speculate that in the islets there is an enzyme system which is very sensitive to certain oxidizing agents. When this enzyme system is inactivated the islets are destroyed and diabetes results. Goldner and Gomori (10) have criticized this general viewpoint on the basis that administration of other oxidizing agents does not produce diabetes. However, there may be a selective action similar to that of tetrathionate on the proximal tubular cells of the kidney (11). These authors point out that tetrathionate has a definite effect on the tubules but no action on the pancreas. Therefore, oxidizing agents may be specific in their biological action. The fact that other oxidizing agents of the same power as alloxan do not produce diabetes is not a conclusive argument against alloxan acting through an enzyme system in the islets of Langerhans, presumably one containing a sulfhydryl group.

The author wishes to thank Mrs. F. P. Lively for her technical aid in these experiments.

CONCLUSIONS

1. Alloxan inhibits the glycolysis of glycogen to lactate in frogs' muscle extract.
2. The degree of inhibition is proportional to the concentration of alloxan.
3. The inhibition of glycolysis produced by alloxan is reversed by cysteine.

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THE RELATION OF O_2 IN BONE MARROW BLOOD TO POST-HEMORRHAGIC ERYTHROPOIESIS[1,2]

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The primary stimulus for erythropoiesis in the adult mammal is generally considered to be anoxia. Since the red bone marrow is well established as the site of red blood cell formation, it is inferred that anoxia of the bone marrow is the specific requirement for the initiation of erythropoiesis. The evidence for this is derived from the association of erythropoiesis with the various types of anoxia. Thus, the well known increase in red blood cells which occurs during a sojourn at high altitude is the result of a low arterial pO_2 (1), whereas the compensatory red blood cell production of chronic hemorrhage, chronic CO inhalation and certain anemias is associated with low or defective O_2 capacity (see 2). It is assumed that the changes in peripheral blood O_2 are paralleled by similar changes in the bone marrow blood. The clearest picture of marrow circulation is obtained from Doan's injection studies (3). The impression gained from a view of the swollen venous sinusoids and numerous large veins is that blood flow through marrow must be slow enough to approach stagnation. Thus, any tendency toward subnormal pO_2 in peripheral blood, as encountered at high altitude or immediately following hemorrhage, might well be accentuated in bone marrow blood. So far as we are aware no direct measurements of the O_2 in bone marrow blood appear in the literature. For this reason the present study was undertaken. Simple hemorrhage was used for the production of an erythropoietic stimulation of bone marrow. During the period of regeneration various determinations of O_2 were performed on blood drawn from the red bone marrow of normal, unanesthetized dogs.

METHODS. *Animals.* Nineteen adult, mongrel dogs, 9 of which were males, were used in this study. The body weights ranged from 6.8 to 14.0 kgm. Their diet was composed of "Friskies"[3] and water *ad libitum.*

Bone puncture. A 16 gauge Osgood bone needle with locking stylet was used. For ease of manipulation a short brass handle was secured to the stylet. The needle was cleansed with alcohol before use. The proximal end of the humerus was selected as the site of routine puncture because this portion of the bone contains red marrow and lies only a few millimeters beneath the skin. After clipping the hair and swabbing the skin over the humerus with alcohol, the skin and subcutaneous tissues overlying the periosteum were infiltrated with approximately 0.2 cc. of 1 per cent procain. The Osgood needle was held

[1] A preliminary report of this work appears in Fed. Proc. 6: 114, 1947.

[2] Aided by a grant from the Josiah Macy Jr. Foundation.

[3] Carnation Co., New York City.

parallel to the long axis of the bone and thrust through the skin and periosteum of the greater tubercle of the humerus by imparting torque to the needle. With only moderate pressure the needle was pushed 2.5 cm. into the proximal end of the bone. The adjacent upper half of the surgical neck of the humerus was avoided as a puncture site because of its numerous vascular foramina. At different times samples were obtained from the same bone by slightly varying the site and angle of penetration. In any one bone, punctures were made usually at intervals of 1 week. Right and left humeri were employed alternately, an individual bone being punctured no more than 5 times. Bone puncture has been performed in some 40 dogs; evidence of temporary trauma has been observed in only two instances. Similar punctures have been carried out in four different bones of dogs as well as in the humeri of cats, rabbits, and rats.

When the puncture had been made and the stylet withdrawn, blood soon appeared in the cup of the needle and usually overflowed at the rate of a drop every 5 to 20 seconds. By attaching a syringe, large quantities of blood could be slowly withdrawn. Fat droplets were observed in bone blood samples but never bone spicules nor other fragments of tissue. Blood so obtained was, in most instances, similar to jugular venous blood in O_2 content and hematocrit value. A large number of immature blood cells were observed in Wright stained smears of bone blood, and their numbers far exceeded those found in jugular venous blood even after as much as 1 cc. of blood had been removed from the bone needle.[4] The removal of larger amounts of blood from the humerus than could conceivably be contained in the bone indicates that such samples must be diluted with peripheral blood. To reduce the possibility of dilution the major portion of analyses were performed on the first 0.15 cc. of blood appearing in the cup of the needle.

Analytical methods. O_2 saturation of blood was calculated from O_2 content and capacity measurements obtained by the use of micro methods. The O_2 content was estimated on 0.04 cc. of blood using the syringe technique of Roughton and Scholander (4). O_2 capacity was also measured on a 0.04 cc. sample by modifying the Roughton and Scholander method (5). CO_2 content was determined manometrically (6), using 0.10 cc. of blood. Direct measurement of O_2 and CO_2 tensions in blood were carried out on 1 cc. samples as described by Riley et al. (7). Duplicate determinations, when made, yielded maximum differences of 6 mm. Hg for pO_2 and somewhat less for pCO_2. An occasional pH measurement of blood was obtained with the glass electrode on 0.1 cc. of blood diluted anaerobically with saline to 1.0 cc.

Hematocrit values were estimated with Van Allen (8) hematocrit tubes which require less than 15 cu. mm. of blood. The diluent used was isotonic sodium oxalate. Erythrocyte and leucocyte enumerations were carried out in the usual manner. Figure 1 presents a correlation between hematocrit values and red blood cell counts of 10 animals, and figure 2 relates hematocrit and O_2 capacity values in 11 animals. Reticulocyte percentages were obtained by

[4] The authors wish to thank Dr. Peter Orahovats for his kindness in performing the hematological examinations.

counting 1000 cells on a dried smear after vital staining with brilliant cresyl blue. In certain experiments plasma volume was estimated by means of the blue dye, T-1824 (9).

Sampling. Blood samples were drawn directly from the cup or syringe end of the needle inserted without stasis in jugular vein or marrow substance. Anaerobiosis was accomplished by placing the tip of the Roughton-Scholander pipette beneath the surface of the blood advancing into the cup. This process required only a few seconds, and no evidence of atmospheric contamination was ever observed (see also 10). Once the pipette was filled, its contents were delivered into the analyzing syringe and the analysis started in less than a minute. Coagulation was prevented by using air dried pipettes which had previously been rinsed with a 1 per cent heparin solution.

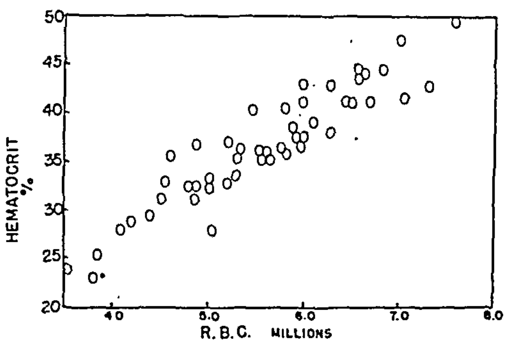


Fig. 1

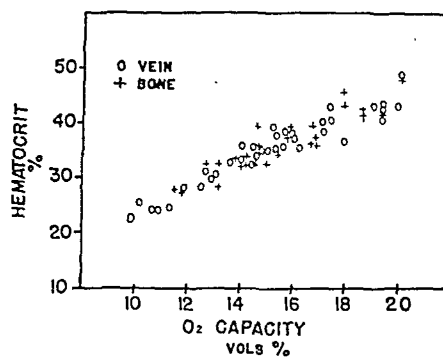


Fig. 2

Fig. 1. The relation of the red blood cell count to the hematocrit value (Van Allen). Data from 10 dogs.

Fig. 2. The relation of O_2 capacity (micro-method) to the hematocrit value (Van Allen). Data from 11 dogs.

Procedure. The control determination consisted of one or two sets of measurements. Both bone and jugular venous blood samples were taken from animals which had not been fed for some 18 hours and which had been lying quietly on an animal board for at least 15 minutes.

Twenty-eight to 32 per cent of the blood volume was removed from a needle inserted in the femoral artery. In 2 instances the blood volume was determined, but in the remainder a blood volume of 95 cc. of whole blood per kgm. of body weight was assumed. Hemorrhage was completed in less than 10 minutes.

Within 20 to 40 minutes from the completion of hemorrhage jugular venous and bone blood samples were removed and analyzed as in the control period. O_2 content and capacity determinations together with hematocrit values and red blood cell counts were determined on most of the animals. In others, CO_2 content, pH, direct pO_2, and direct pCO_2 were estimated on both bone and venous bloods while white blood cell counts, reticulocyte percentages, and total blood volume were measured using venous blood samples.

A second post-hemorrhagic series of measurements was made 20 to 24 hours

later. Determinations were then repeated at 3 to 6 day intervals for about 3 weeks. The study of hemorrhaged animals was rarely continued to the point at which the erythrocyte levels had returned to those of the controls. Since we were concerned with the relation of O_2 in bone marrow blood to the initiation of erythropoiesis, the first two weeks after bleeding were most important.

RESULTS. *Normal bone blood values.* In view of the lack of information concerning blood gas values of blood circulating through normal bone marrow, various measurements made during the control period have been assembled and are presented in table 1. These data will be discussed later.

Erythrocyte concentration. In 11 dogs studied for 19 to 26 days after hemorrhage, the red blood cell count fell rapidly and approximately one day later had reached its lowest level. A gradual increase was observed over the remainder of the period and at 21 days the count of several animals had returned to that of

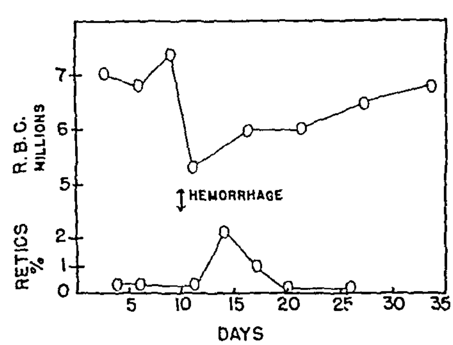


Fig. 3

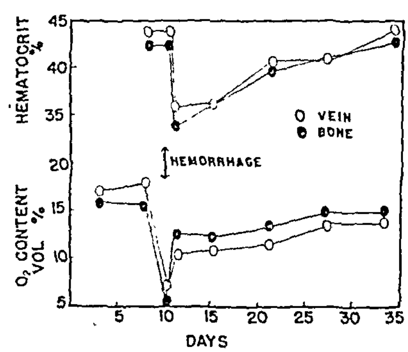


Fig. 4

Fig. 3. The response of the jugular venous red blood cell count and of reticulocyte percentage to a 30 per cent hemorrhage.

Fig. 4. The effect of 30 per cent hemorrhage on the hematocrit value (Van Allen) and O_2 content of jugular venous and bone marrow blood. The first post-hemorrhagic values were obtained 30 minutes after bleeding. At this time the hematocrit percentage is hardly altered but the O_2 content has decreased considerably.

the control level. As an index of active regeneration, venous reticulocyte percentages were measured in three animals. A sharp but transient rise in the reticulocyte level occurred from 5 to 8 days after bleeding. The red blood cell count and reticulocyte percentage curves of a typical bled dog are shown in figure 3.

Changes observed in the hematocrit values were similar to those shown by red blood cell counts. Hematocrit measurements were made on both venous and bone marrow blood and were followed more closely than was the erythrocyte enumeration. No consistent difference was observed between hematocrit values of bone and jugular venous bloods of 15 normal dogs obtained during the control period. At this time average venous and bone blood readings were 40.7 and 41.0 (table 1), respectively. In samples removed within the first hour after bleeding, the values had decreased by 1 to 5 hematocrit divisions (fig. 4). Under the adopted schedule, minimal hematocrit readings, 7 to 18 divisions below con-

trol, were observed 24 hours after the blood loss indicating that compensating dilution had occurred. Readings obtained 2 to 3 days later were either identical with the previous one or slightly greater. In the ensuing 3 weeks, hematocrit values rose steadily. In general, hematocrit measurements of bone and venous bloods were similar.

Blood volume determinations were made on 2 dogs, before and 3 days after the standard hemorrhage. Typically, in 1 animal the loss of 32 per cent of total blood volume resulted in a decrease in hematocrit value and red cell volume while dilution of the plasma with tissue fluid occurred. Three days after bleeding the total blood volume had been restored to 92 per cent of control value.

Oxygen content, capacity, and saturation. During the control period the O_2 content of bone and jugular venous bloods agreed within the error of the methods used (table 1). The average value was 13.7 vol. per cent. Twenty to 40 minutes after hemorrhage, bone and venous samples showed striking decreases in the

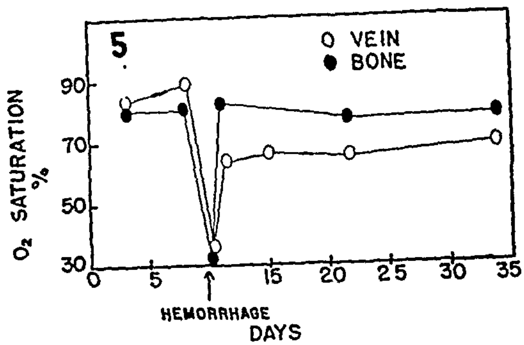
TABLE 1

Control determinations carried out upon bone and venous bloods of normal dogs

	VEIN				BONE			
	Number of samples	Number of dogs	Average	Range	Number of samples	Number of dogs	Average	Range
O_2 vol. %	20	14	13.7	8.8–18.5	20	14	13.72	9.5–19.0
Hct. %	31	15	40.7	30.9–49.5	24	15	41.0	33.0–49.3
O_2 cap. vol. %	15	10	18.3	13.6–20.4	8	8	17.03	14.6–20.3
O_2 sat. %	9	9	75	59–85	8	8	78	65–92
pO_2 mm. Hg	10	5	54	42–72	10	5	65	55–73
pCO_2 mm. Hg	11	5	42.1	35–50	11	5	43.8	36–56
CO_2 vol. %	4	2	40.4	39.6–41.5	3	2	38.8	37.5–41.2
pH	1	1	7.35		1	1	7.35	

O_2 content (fig. 4) which amounted to a reduction of 5 to 12 vol. per cent below the control level. This represents a loss of more than half the normal amount of O_2 carried by a unit volume of blood. Concomitant with this marked deficit in O_2 content, the hematocrit value declined only slightly. O_2 capacity of blood which followed a course parallel to that of the hematocrit percentage (see fig. 2) also showed a small decrease 20 to 40 minutes after bleeding. In the immediate post-hemorrhagic period, the blood of vein and bone had retained the major portion of its O_2 carrying capacity, yet the quantity of O_2 present in the blood was halved. Percentage O_2 saturation, which averaged 75 in jugular and 78 in bone blood during the control period, declined within 20 to 40 minutes after hemorrhage to values ranging betwen 18 and 50 in both bone and vein (fig. 5). Although no direct measurements were made, it was suspected that the low O_2 saturation was a result of decreased circulatory rate in the areas studied. An indication of this was observed in the slowing of blood flow from needles in bone substance and vein during sampling.

Twenty-four hours after hemorrhage, the volume of whole blood lost by bleeding had been replaced by tissue fluid. The increased plasma volume and decreased hematocrit value have already been noted. In bone and venous blood samples taken at this time, O_2 content had risen 1 to 8 vol. per cent above the



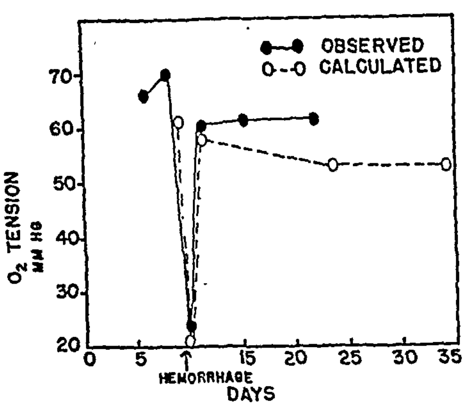


Fig. 6

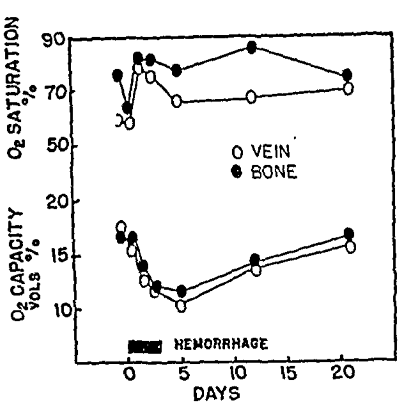


Fig. 7

Fig. 5. The percentage O_2 saturation of both jugular venous and bone marrow blood is markedly decreased immediately after 30 per cent hemorrhage. The values for bone blood rapidly attain the control level while those of jugular venous blood remain for some time below this range.

Fig. 6. The effect of 30 per cent hemorrhage upon the O_2 tension of bone marrow blood. The observed points were determined by the method of Riley et al. (7). The calculated values were obtained by applying the O_2 saturation determinations to Barcroft's O_2 dissociation curve for dog's blood (11).

Fig. 7. The effect of the removal of 30 per cent of the red cell volume by 6 equal hemorrhages over a period of 3 days upon the O_2 saturation and the O_2 capacity of bone marrow and jugular venous blood.

values attained a few minutes after hemorrhage (fig. 4). During the following 3 weeks, O_2 content gradually approached the control level. In this period, bone marrow blood consistently yielded an O_2 content 2 to 4 vol. per cent above that of jugular venous blood.

The percentage O_2 saturation on bone and venous bloods, which immediately after bleeding was markedly reduced, had increased dramatically to approach

control levels 24 hours later (fig. 5). Although the O_2 content remained low, the reduction in O_2 capacity by dilution of the blood raised the O_2 saturation. Bone samples generally showed saturation measurements within the control range, 20 to 24 hours following the bleeding. Venous blood, while much increased over its previous level, remained below the control range for 2 to 3 weeks and occasionally longer. Blood samples were obtained more readily from both vein and bone a few days after bleeding than was the case immediately following hemorrhage. On the basis of routine inspection, blood flow from inserted needles was much accelerated and often exceeded the control velocity.

Oxygen tension. In order to evaluate O_2 tension of bone marrow blood, percentage oxy-hemoglobin values were applied to the O_2 dissociation curves for dog blood constructed by Barcroft (11). Although this curve was not prepared under conditions identical with those of this study, it permits a first approximation of the pO_2. According to the Barcroft curve the average control O_2 saturation of 78 per cent (8 dogs) gives a pO_2 value of 57 mm. Hg. Figure 6 shows O_2 tensions derived in this manner. From this graph it may be observed that the pronounced depression of pO_2 in the first post-hemorrhagic hour has almost completely disappeared in bone marrow blood 24 hours later.

The pO_2 estimates obtained indirectly from O_2 saturation measurements are compared with those obtained by *direct* determinations of O_2 tensions using the Roughton-Scholander syringe as described by Riley et al. (7). Control values on 5 animals are listed in table 1. The average of 10 analyses of bone blood was 65 mm. Hg with a range of 55 to 73 mm. Hg. The average pO_2 determined directly, therefore, agrees reasonably well with the value of 57 mm. Hg obtained indirectly. More important in this study than the absolute values of O_2 tension were the relative changes following blood loss. In this respect, the pO_2 changes estimated directly closely parallel those derived from the O_2 dissociation curve (see fig. 6).

Carbon dioxide. A detailed study of CO_2 and pH is beyond the scope of the present investigation. A few exploratory measurements, however, were made and control values are included in table 1.

Length of stagnant anoxia. The foregoing sections have indicated that an abrupt depression of the O_2 saturation occurred in bone blood immediately following a 30 per cent hemorrhage. Twenty-four hours later the O_2 saturation had returned to the control range. To determine more precisely the duration of low O_2 saturation, measurements of bone blood were performed at hourly intervals after bleeding. This procedure was carried out in 2 animals. During this period, blood dilution proceeded gradually at a uniform rate as noted from the declining hematocrit and O_2 capacity values. After the immediate decrease, O_2 content rose slowly. The O_2 saturation which was markedly depressed at the close of hemorrhage returned to normal in the 2 animals in 3 and 5 hours, respectively.

Elimination of stagnant anoxia. In an attempt to reduce or to eliminate the low O_2 saturation following hemorrhage, 2 dogs were bled so that 30 per cent of the calculated red cell volume was removed. This was accomplished by drawing blood in 6 equal quantities over a period of 3 days (fig. 7). Blood dilution, of

course, makes necessary the removal of more than 30 per cent of the total blood volume in order to reduce the red cell volume by 30 per cent. The usual control determinations were made prior to the first of the series of 6 hemorrhages, and thereafter measurements were repeated 20 to 40 minutes after every other bleeding. No marked drop in percentage of oxygen saturation was observed in measurements of bone and venous bloods, either during or following the periods of divided hemorrhages. In 1 animal a single determination of bone blood O_2 saturation showed 12 per cent decrease and in the other dog a similar change occurred in one of the samples of venous blood. In all other instances the values coincided with or exceeded the control levels. Dogs were studied for the customary period of 3 weeks; and recovery curves of hematocrit readings, O_2 capacities, and O_2 content as well as percentage O_2 saturations were found to parallel those obtained from animals which had been subjected to the single standard hemorrhage. For all practical purposes, the temporary post-hemorrhagic depression of percentage O_2 saturation was abolished by the repeated small hemorrhages, yet the evidence indicates that erythropoiesis proceeds as after a single massive bleeding.

DISCUSSION. *Source of bone blood.* Although at present no definite statement can be made concerning the exact source of bone blood samples, certain inferences can be drawn. For example, dissection and histological examination demonstrate that the tip of the bone sampling needle rests well within the confines of red marrow. This statement is substantiated by the presence in the drawn blood of large numbers of immature erythrocytes and leucocytes as well as megakaryocytes and fat droplets. Additional information is provided by blood gas measurements. The O_2 saturation of normal bone blood which is similar to that of jugular venous blood is well below that of arterial blood. Thus, on the basis of O_2 saturation, we consider that bone blood is venous in character.

Blood samples can be obtained from punctures made in different regions of the head of the humerus as well as from several other bones. As mentioned previously, blood has been drawn from the bone marrows of several different animals commonly used in the laboratory. These statements suggest that blood is not removed from any single specialized vessel, but rather from a diffuse series or network of vessels common to diverse bones and animals. Marrow venous sinusoids would conform to the latter possibility since they are numerous, wide and highly typical of bone marrow.

Successively drawn bone samples have been studied in a series of 6 animals. The average arterial O_2 content exceeded that of bone blood by 4.0 vol. per cent. Comparison of the O_2 content of the first drop of blood from bone with the O_2 content of a second drop of blood obtained from the same bone needle after 3 to 10 cc. of blood had been aspirated and discarded, showed that no significant increase in O_2 content occurred. The failure of the O_2 content of the second drop of blood to approach the arterial O_2 level suggests that even when 10 cc. of blood are withdrawn, the latter portion is obtained from venous rather than from arterial sources.

That large bone blood samples must in part be derived from extramedullary

vessels is indicated from the following measurement. The blood contained in the humerus was calculated from the carbon monoxide capacity of an aqueous extract of the crushed bone. According to this determination the maximal blood volume of a single dog's humerus was roughly estimated as 10 per cent on wet-weight basis. Volumes of blood which may easily be withdrawn far exceed this first approximation of the total quantity contained in the bone.

In this study the major portions of all analyses were performed on the first 0.15 cc. of blood which welled into the needle. During its passage through marrow substance the bone needle probably ruptures veins and venous sinusoids, dislodging clusters of parenchymal cells and fat globules. Material thus liberated is forced into the needle by the pressure of the blood. We believe that under these circumstances, small samples (0.1 to 0.2 cc.) are not contaminated by extramedullary blood, but rather that they are representative of the blood present in bone marrow.

Post-hemorrhagic anoxia. Hemorrhage is a common method of producing erythropoietic stimulation. As is well known, blood loss is followed by a transient period of stagnant anoxia, which is succeeded by a state of anemic anoxia. The interval of stagnant anoxia lasts the same length of time in the bone marrow as in the jugular venous blood. This is surprising, for marrow is generally visualized as a region of extremely sluggish blood flow.

If low O_2 saturation of bone marrow blood be the stimulus for erythropoiesis, its influence must be exerted in from 3 to 5 hours. According to the data obtained from studies carried out at high altitude and in the low pressure chamber, short periods of low O_2 saturation and tension appear insufficient to accelerate red blood cell production (12). However, it can be postulated that even a 3 hour period of stagnant anoxia might act as a "trigger mechanism" in initiating erythrocytic regeneration. By the use of 6 small hemorrhages, spaced over a period of 3 days, the usual reduction in O_2 capacity was achieved with little if any dimunition of percentage O_2 saturation of bone and venous blood. Nevertheless, regeneration of lost erythrocytes proceeded in a manner similar to that following a single 30 per cent hemorrhage. The transient stagnant anoxic phase appears to be of small importance since its abolition produces no discernible difference in regeneration rate. It is interesting to note that Warren's (14) metabolic studies of bone marrow made in vitro offer no support for the concept of erythroid cell stimulation by lowered O_2 tension.

This conclusion suggests that if anoxia be the stimulus for erythropoiesis it must act during the period of anemic anoxia. At this time the red cell count, the hematocrit value, the O_2 content and the O_2 capacity of bone and venous bloods are reduced below the control values. The O_2 saturation and O_2 tension, however, lie within the ranges of the control determinations. The red cell count and the hematocrit changes are probably not concerned with marrow stimulation for these are not reduced by a degree of carbon monoxide saturation which eventually produces polycythemia (13). These observations suggest that the occurrence of post-hemorrhagic erythropoiesis at sea level O_2 pressure is not dependent upon a decrease in erythrocyte numbers, but may be related to a deficiency in the O_2 content and the O_2 capacity of the blood.

Relation of red cell recovery to blood reservoirs. The results reported in this paper are concerned only with the changes which follow a single hemorrhage amounting to 30 per cent of the circulating blood volume or to the response shown by dogs in which 30 per cent of their circulating red cells have been removed over a period of several days. Under these circumstances some of the recovery in hematocrit values, red cell counts, etc., may have occurred at the expense of blood reservoirs. Since the same pattern of recovery was seen following hemorrhage in 2 splenectomized dogs we are not inclined to attribute undue importance to the rôle of the blood reservoirs. Moreover, the reticulocytosis shown in figure 3 suggests increased activity in the bone marrow. We are aware of the fact that the removal of 30 per cent of the red cells does not produce maximal stimulation of the bone marrow. Nevertheless, hemorrhages of this magnitude can be followed by recovery of the red cell mass without changes in the O_2 tension or saturation of bone marrow blood.

SUMMARY

A technique for the removal of blood samples from the interior of red bone marrow of the unanesthetized dog is described.

Blood so obtained was studied during a period of erythropoietic stimulation induced by a single 30 per cent hemorrhage. O_2 content, O_2 capacity and hematocrit value of bone marrow and jugular venous blood are decreased after hemorrhage. A gradual recovery occurs during the following three weeks indicating red blood cell regeneration. O_2 saturation and O_2 tension although markedly decreased immediately after a single hemorrhage return to control levels within 3 to 5 hours where they remain during the subsequent period of observation.

Abolition of the transient decrease in O_2 saturation can be accomplished by repeated removal of small quantities of blood over a period of several days. Under these circumstances, the erythropoietic response is the same as that following the single hemorrhage.

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INFLUENCE OF ENVIRONMENTAL TEMPERATURE AND POSTURE ON VOLUME AND COMPOSITION OF BLOOD[1]

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It has been established that blood volume of man increases during exposure to heat and decreases during exposure to cold (1–3) but a description of the details of this phenomenon is lacking. Experimental study is rendered difficult by the necessity for confining human subjects to a single room for days or weeks, by the complex manner in which environmental temperature influences blood volume, and by variation of the volume and composition of the blood with the season of the year (4).

This report is limited to the effects produced by exposure (to either heat or cold) of about one week. It is possible that further important changes occur on longer exposure. Seasonal influences have been avoided to a great extent since a separate investigation of this phenomenon has enabled us to perform our experiments at times of the year suitable to our purpose.

During the course of these experiments, it was found that circulating plasma protein increases progressively in amount during the day (waking) and decreases during the night (sleeping). Further study indicated that posture is the factor chiefly responsible for these changes.

EXPERIMENTAL METHODS AND PROCEDURES. *Analytical methods.* The differential electric photometer of this laboratory (4) was employed to determine concentrations of carboxyhemoglobin and of total hemoglobin. Calibration for optical density readings was made using grey glass filters of known transmission (determined by Bureau of Standards). A detailed description of this instrument has been published (4).[2]

Percentage saturation of hemoglobin with carbon monoxide is calculated from determination of transmission (T) of the green (λ 546) and yellow (λ 578) lines of mercury, employing the relationship given below.

$$\frac{-\log T_{546}}{-\log T_{578}} = \frac{e_1 C + e_2(1 - C)}{e_3 C + e_4(1 - C)}$$

Where e_1 and e_2 are the respective extinction coefficients of carboxyhemoglobin and oxyhemoglobin for λ546; e_3 and e_4 are corresponding values for λ 578; and C is the fraction of hemoglobin combined with carbon monoxide.

It is convenient in actual practice to determine the relationships between

[1] This investigation was supported by a Life Insurance Medical Research Fund grant to Prof. H. C. Bazett.

[2] Since this publication, changes have been made in the photometer by Drs. M. E. Maxfield and H. C. Bazett. These were necessitated by alterations in the available mercury vapor lamps. The changes made are indicated in an appendix to this paper.

extinction coefficients rather than actual values. Letting $\frac{e^1}{e_2} = L$; $\frac{e_3}{e_1} = M$; and $\frac{e_4}{e_2} = N$, we have

$$\frac{-\log T_{546}}{-\log T_{578}} = \frac{1 + C(L - 1)}{LMC + N(1 - C)}$$

The value for L, determined using samples of pure carboxyhemoglobin and oxyhemoglobin, is very close to 0.96, which is the value employed by others (4, 5). Values for M and N seem to be dependent upon relatively slight modifications of the apparatus and are changed in the same direction by any such modification. In our experience, values for M and N lie within the limits 0.745–0.747, and 1.104–1.114, respectively. Because of this variability, M and N should be determined at frequent intervals if absolute levels of carboxyhemoglobin are desired. For determination of differences between two samples in concentration of carboxyhemoglobin (as in blood volume estimation) the variability in values for M and N introduces an error of only 1 to 2 per cent (in the estimate of blood volume).

The concentration of total hemoglobin is calculated from determinations of transmission of the green line of mercury (λ 546).[3]

Hematocrits are obtained with tubes (six tubes are used for each determination) which are centrifuged one hour at a speed of 2200 rpm with an arm length of 18 cm. Hematocrit values are multiplied by the factor (0.96) to correct for plasma contained between packed erythrocytes. Values given in the tables are so corrected.

Protein concentrations of serum (or plasma) are estimated routinely from specific gravity determinations according to the technique described by Phillips, Van Slyke et al. (6). The biuret method of Kingsley (7) is also used occasionally.

Preparation of blood samples. For determining concentrations of carboxyhemoglobin and of total hemoglobin, 0.4 cc. of heparinized blood is placed in a 12 cc. centrifuge tube nearly filled with saline saturated with oxygen. After shaking, the tube is centrifuged (10 min.) and the supernatant fluid removed. The tube is then filled to the calibrated level with oxygenated 0.1 per cent Na_2CO_3. The tube is shaken until hemolysis has occurred and again centrifuged (1 hr.) to separate particulate matter. The optically clear supernatant fluid is transferred to a spectrophotometric cell for determination of light transmission. Care is taken with samples that are to be analyzed for carboxyhemoglobin to prevent unnecessary exposure to air with consequent loss of carbon monoxide.

Determination of blood volume. Most of our observations (except those

[3] Values for hemoglobin concentration determined on this instrument are in close agreement with values obtained on the same solutions using a standard spectrophotometer. We are indebted to Dr. D. L. Drabkin of the Department of Physiological Chemistry for analyzing several of our solutions for hemoglobin.

dealing with diurnal or postural effects) were made on subjects in the basal state. The general technique of administering carbon monoxide is similar to that employed previously (4). Following a control blood sample (drawn without stasis from an antecubital vein), carbon monoxide (approximately 90 cc. in 5 to 10 l. of oxygen) is respired for 22 to 24 minutes. A second sample of blood is then taken. The difference between the two samples in carboxyhemoglobin concentration (in per cent) divided into the hemoglobin equivalent[4] of the amount of carbon monoxide that has been absorbed,[5] is considered to represent the total quantity of circulating hemoglobin (Total Hb). From this figure blood volume (B.V.) may be obtained using equation (1), and total protein (Total Pr), using equation (2).

$$(1)\quad \text{B.V.} = \frac{\text{Total Hb}}{\text{Hb}_{\text{conc.}}}$$

$$(2)\quad \text{Total Pr} = \text{Pr}_{\text{conc.}} \times \text{B.V.} \times (1 - 0.96 \text{ hematocrit})$$

These equations may be combined to give equation (3).

$$(3)\quad \text{Total Pr} = \text{Total Hb}\left[\frac{\text{Pr}_{\text{conc.}} \times (1 - 0.96 \text{ hematocrit})}{\text{Hb}_{\text{conc.}}}\right]$$

Letting the reciprocal of the expression in parenthesis equal R, we have

$$(4)\quad \text{R} = \frac{\text{Total Hb}}{\text{Total Pr}} = \frac{\text{Hb}_{\text{conc.}}}{\text{Pr}_{\text{conc.}} \times (1 - 0.96 \text{ hematocrit})}$$

As indicated in equation (4) the ratio (R) of total quantity of circulating hemoglobin relative to total quantity of circulating plasma protein can be determined from data obtained on a single sample of blood. The ratio which is easily determined is useful in indicating whether or not a change in amount of hemoglobin relative to protein has occurred but, of course, does not indicate which substance is involved. For example, the possibility exists that the quantity of both hemoglobin and protein may change in the same direction to such a degree as to leave the ratio unaltered.

Accuracy of determinations. Accuracy of estimates of total hemoglobin depend to a great extent upon the accuracy with which concentrations of carbon monoxide are estimated; however, other factors introduce sporadic errors. With our most precise procedure all values are probably correct to within ± 5 per cent. Estimates of values for the ratio of total hemoglobin to total plasma protein appear to have an accuracy of approximately the same degree.

[4] One and thirty-four one-hundredths cubic centimeters of carbon monoxide under standard conditions (dry at 0°C and 760 mm.) is assumed to combine with one gram of hemoglobin.

[5] Estimated to be 99 per cent of the amount of carbon monoxide introduced into the respiratory apparatus. Purity of stock carbon monoxide is estimated by analyzing for oxygen. If stock carbon monoxide is stored under water, the proportion of oxygen to nitrogen is approximately the same as their proportionate solubilities in water under an atmosphere of air.

Description of experiments. *Environmental studies.* Many of the data reported here were obtained on two young men[6] who were confined to a psychrometric room maintained at the temperature desired. In one experiment carried out during the latter part of July (summer experiment) the room was at first maintained uncomfortably warm (91°F., dry bulb; 82°F., wet bulb) for six days; then the room temperature was lowered and maintained uncomfortably cold (69°F., dry bulb; 60°F., wet bulb) for six days; this was followed by a period of four days in which the room was again maintained at the original uncomfortably warm level. In a second experiment, carried out in the latter part of December and first part of January (winter experiment), the room was at first maintained uncomfortably cold (69°F., dry bulb; 59°F., wet bulb) for two days; this was followed by a warm period (91°F., dry bulb; 83°F., wet bulb) of seven days' duration at the end of which time the room was again maintained at the original uncomfortably cold temperature for one-half day.

Blood volume of each subject was determined on alternate mornings (8–9 a.m.) during these experiments. In the winter experiment, samples of blood were also taken each afternoon (4–5 p.m.) and on those mornings that determinations of blood volume were not made.

Diurnal studies. The data obtained on mornings and evenings during the winter experiment (see above) were supplemented by determinations of volume and composition of blood of several laboratory workers at night (4–5 p.m.) and the following morning (fasting) after arriving at the laboratory. Subjects were instructed to avoid extremes of temperature between the two determinations; otherwise environmental temperature was not controlled.

Postural studies. Preliminary postural studies were made during the course of experiments designed to study the influence of environmental temperature on blood volume (summer experiment). In these studies, subjects were required to stand for one half hour (following routine blood volume determination in the resting, fasting state) at which time blood samples were again drawn. Since subjects remained connected with the respiratory apparatus during this period, these additional blood samples were analyzed for carboxyhemoglobin to determine whether standing caused additional hemoglobin to appear in the circulatory system.

In other postural studies, blood samples from fasting subjects were obtained at intervals throughout two periods of four hours. In one of these two periods (scheduled a day or two apart), subjects lay completely at rest; in the other period, subjects sat passively in bed with the back supported. Reading was not allowed in either experiment. Room temperature was maintained constant at the same comfortable level throughout the experiments.

The remaining studies on posture were of longer duration (24–34 hrs). In one of these experiments, the time was divided into five periods of seven hours in which the subject was alternately lying at rest (reading was allowed) or sitting. In a second experiment, the subject lay at rest or slept for 12 hours and then sat

[6] One subject was an American-born Japanese (Y) (age, 21; ht., 160 cm., wt., 51.1 kgm.); the other a native Porto Rican (R) (age, 23; ht., 178 cm., wt., 59.0 kgm.).

for 12 hours. In a third experiment the subject remained in bed (reading or sleeping) for 34 hours.

Control studies. Known amounts of plasma protein[7] were infused in four subjects to determine the exactness with which changes in quantity of circulating plasma protein were estimated. Infusions were made at rates of 4–10 cc. per minute. In most instances determinations of blood volume were made before and subsequent to the infusion. Blood samples, taken at suitable intervals, indicated changes in blood composition.

RESULTS. *Environmental studies.* During the first six (warm) days of the summer experiment there was very little change in hemoglobin concentration or serum protein concentration (fig. 1). This was expected since the subjects had been exposed previously for several weeks to warm summer weather. When the room was cooled at the end of the sixth day, both hemoglobin and serum protein quickly became more concentrated and remained so throughout the cold period. During the first part of the cold period, there was a decrease in total quantity of circulating serum protein, as shown by the fact that the ratio of total hemoglobin to total serum protein increased without appreciable change in amount of total hemoglobin. On rewarming the room, hemoglobin concentration, protein concentration and total amount of protein approached levels existing previous to cooling.

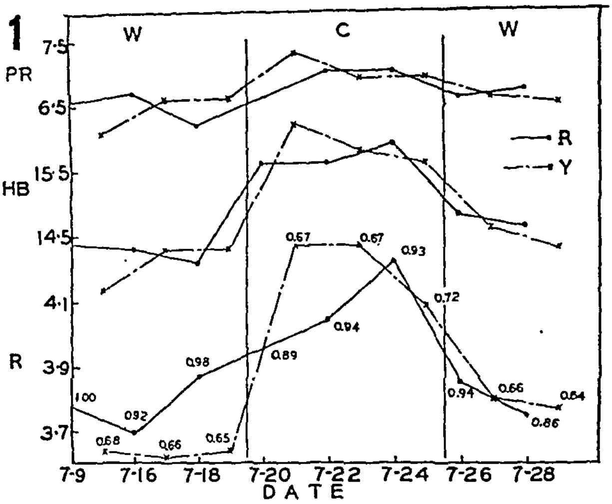
The winter experiment began with a cold period (two days) during which time the ratio of total hemoglobin to total serum protein attained approximately the level that existed during the cold period of the summer experiment. In the following warm period (7 days), protein concentration and hemoglobin concentration declined quickly and remained at lower levels (fig. 2). The quantity of circulating plasma protein increased rapidly at first and then more slowly throughout this period as indicated by the decrease in ratio of total hemoglobin to total serum protein without change in quantity of total hemoglobin (fig. 2). At the end of the final cold period (one-half day) all values tended to approach levels existing during the first cold period.

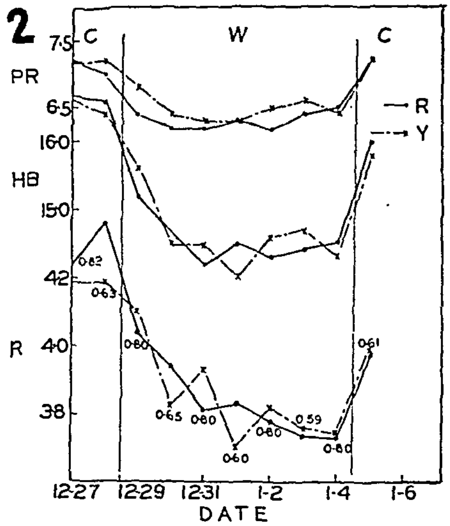
Diurnal studies. Analysis of blood samples, taken daily at 4–5 p.m. during the winter experiment, showed a consistently lower (4 per cent) ratio of total hemoglobin to total serum protein than that shown in the morning (10 a.m.) (table 1). However, data were not obtained at this time which permitted a decision as to whether total hemoglobin had decreased in amount during the day or whether serum protein had increased in amount. In a later study, determinations of total hemoglobin were made at night and the following morning on several subjects who were allowed to follow their usual daily routines. There was no significant diurnal variation in amount of hemoglobin (table 2). In these experiments the average ratio of total hemoglobin to total serum protein at night was 5 per cent lower than in the morning.

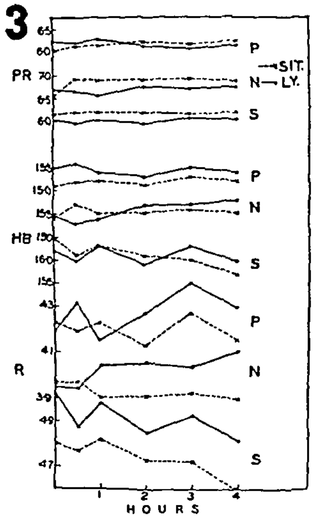
Postural studies. Although the ratio of total hemoglobin to total serum protein was not altered detectably[8] within one-half hour after changing from

[7] Sharp and Dohme, Philadelphia, kindly donated the plasma.

[8] The well-known alterations in concentrations of hemoglobin and plasma protein, which occur on changing posture, were always observed in our experiments.







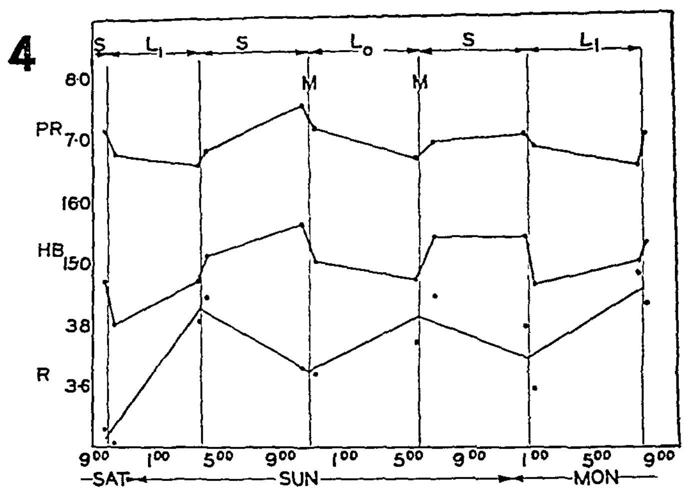


Fig. 1. Influence of environmental temperature on protein concentration (*Pr*), hemoglobin concentration (*Hb*), and the ratio (*R*) of total quantity of hemoglobin to total quantity of serum protein (summer experiment). The numerals entered near each point in the graphs of the ratios are values (in kgm.) for total hemoglobin (determined by carbon monoxide). The psychometric room was at first maintained warm (*W*); subsequently the room was kept cold (*C*); and finally the original warm conditions were reproduced. The first warm period was interrupted on July 10 by apparatus failure. The experiment was begun again on July 16 and was completed without further interruption. Subjects were R and Y.

Fig. 2. Influence of environmental temperature on protein concentration (*Pr*), hemoglobin concentration (*Hb*), and the ratio (*R*) of total quantity of hemoglobin to total quantity of serum protein (winter experiment). The numerals entered near each point in the graphs of the ratios are values (in kgm.) for total hemoglobin (determined by carbon monoxide). Environmental conditions are indicated by *C* (cold) and *W* (warm). Subjects were R and Y.

Fig. 3. Influence of posture on protein concentration (*Pr*), hemoglobin concentration (*Hb*), and ratio (*R*) of total quantity of hemoglobin to total quantity of serum protein. These data were obtained on three male subjects (*N*, *P*, *S*) who sat passively in bed or, in companion experiments, lay down for four hours in a room maintained at a comfortable temperature. Subjects were fasting and lay down for one-half hour before the experiments started. The two experiments (sitting and lying) on each subject were carried out within an interval of two to four days.

Fig. 4. Influence of posture on protein concentration (*Pr*), hemoglobin concentration (*Hb*), and ratio (*R*) of total quantity of hemoglobin to total quantity of serum protein. The subject (*R*) alternately sat (*S*) or lay down (*Lo*) (L_1 indicates the subject slept most of the time) for seven hours for five consecutive periods. The time of meals (*M*) is indicated.

TABLE 1

Influence of time of day on hemoglobin concentration, protein concentration, hematocrit and ratio (R) of total quantity of hemoglobin to total quantity of serum protein.

Data were obtained during the *winter experiment* in the morning (a.m.) before breakfast with the subjects lying at rest and in the evening (p.m.) before dinner with the subjects either in the sitting or lying position. Mean values for hemoglobin and protein concentrations and for hematocrits are not given since evening samples were not obtained under uniform postural conditions.

DATE	Hb CONC.		HEMAT.		Pr CONC.		R		
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	p.m./a.m.
Subject R									
12–27	16.7	16.4	0.453	.453	7.2	7.2	4.24	4.17	0.98
12–28	16.6	16.2	0.457	.447	7.0	7.0	4.37	4.19	0.96
12–29	15.2	15.7	0.413	.443	6.4	6.8	4.05	4.07	1.00
12–30	14.6	14.9	0.404	.413	6.2	6.7	3.95	3.79	0.96
12–31	14.2	14.4	0.399	.397	6.2	6.8	3.81	3.51	0.92
1–1	14.5	13.5	0.400	.372	6.3	6.2	3.84	3.47	0.90
1–2	14.3	14.7	0.390	.404	6.2	6.8	3.78	3.63	0.96
1–3	14.4	13.9	0.398	.383	6.4	6.3	3.74	3.58	0.96
1–4	14.5	15.1	0.403	.417	6.5	7.1	3.74	3.65	0.98
Subject Y									
12–27	16.6	15.8	0.452	.449	7.2	7.1	4.21	4.04	0.96
12–28	16.4	16.1	0.457	.456	7.2	7.3	4.20	4.05	0.96
12–29	15.6	14.5	0.442	.410	6.8	6.3	4.11	3.90	0.95
12–30	14.5	14.9	0.408	.423	6.4	7.0	3.83	3.69	0.96
12–31	14.5	14.2	0.415	.403	6.3	6.5	3.93	3.66	0.93
1–1	14.0	14.6	0.399	.413	6.3	6.9	3.70	3.61	0.98
1–2	14.6	14.2	0.413	.403	6.5	6.4	3.83	3.72	0.97
1–3	14.7	14.1	0.409	.416	6.6	6.9	3.77	3.50	0.93
1–9	14.3	14.0	0.404	.396	6.4	6.4	3.75	3.62	0.97
Mean							3.94	3.77	0.96

TABLE 2

Influence of time of day on hemoglobin concentration, protein concentration, hematocrit, ratio of total quantity of hemoglobin to total quantity of serum protein, and total quantity of hemoglobin (determined by carbon monoxide).

Data were obtained in the morning (a.m.) and evening (p.m.) on several laboratory workers (see text). Determinations were made with subjects recumbent. Values for total hemoglobin are in kilograms.

SUBJ.	Hb CONC.		HEMAT.		Pr CONC.		R			TOTAL Hb		
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	p.m./a.m.	a.m.	p.m.	p.m./a.m.
P	15.3	15.4	0.420	0.425	6.2	6.3	4.26	4.25	1.00	0.82	0.77	0.94
S	15.7	15.6	0.448	0.451	6.0	6.4	4.74	4.44	0.94	0.80	0.79	0.99
R	14.8	13.4	0.403	0.392	6.4	6.4	3.87	3.44	0.89	0.78	0.82	1.05
P	15.2	15.1	0.417	0.418	6.3	6.3	4.14	4.12	1.00	0.84	0.79	0.94
S	16.2	16.3	0.469	0.477	6.0	6.5	5.08	4.80	0.94	0.78	0.82	1.05
Y	16.1	15.1	0.450	0.422	6.7	6.5	4.37	4.02	0.92	0.64	0.65	1.02
S	16.1	15.8	0.464	0.452	6.1	6.0	4.92	4.80	0.98	0.86	0.83	0.97
Mean	15.6	15.2	0.439	0.434	6.2	6.3	4.48	4.27	0.95	0.79	0.78	0.99

a lying to a standing position (table 3), evidence suggesting that this ratio was influenced by posture was obtained in studies lasting four hours (fig. 3). In all three subjects studied, this ratio was less at the end of the period of passive sitting than at the end of the period of lying down. Confidence in this result

TABLE 3

Influence of change in posture on hemoglobin concentration, protein concentration, hematocrit ratio of total quantity of hemoglobin to total quantity of serum protein, and total quantity of hemoglobin (determined by carbon monoxide).

Data were obtained in the summer experiment on subjects lying in the basal state and after standing one-half hour. Values for total Hb are in kilogram.

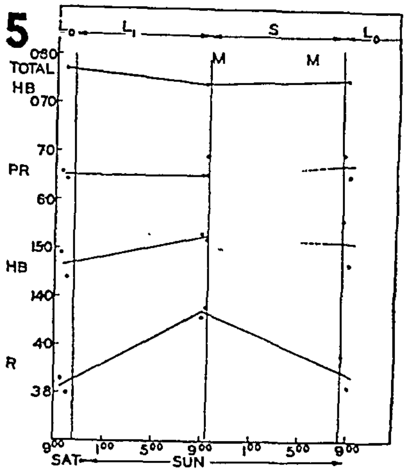
DATE	Hb CONC.		HEMAT.		Pr CONC.		R			TOTAL Hb		
	Lyng.	Stdg.	Lyng.	Stdg.	Lyng.	Stdg.	Lyng.	Stdg.	Stdg./Lyng.	Lyng.	Stdg.	Stdg./Lyng.
						Subject R						
7–9	14.4	15.7	0.423	0.451	6.6	7.5	3.78	3.81	1.01	1.03	0.99	0.96
7–16	14.3	15.8	0.422	0.445	6.7	7.6	3.69	3.75	1.02	0.87	0.98	1.13
7–18	14.1	15.2	0.412	0.442	6.2	7.3	3.87	3.73	0.96	0.98	1.01	1.03
7–20	15.6	16.4	0.457	0.476		7.7*		4.07*		0.88	0.90	1.02
7–22	15.6	16.6	0.450	0.492	7.0	8.0	4.05	4.08	1.01	0.94	0.94	1.00
7–24	15.9	16.7	0.462	0.485	7.0	8.0	4.22	4.05	0.96	0.90	0.97	1.08
7–26	14.8	16.1	0.418	0.473	6.6	7.6	3.86	4.02	1.04	0.93	0.95	1.02
7–28	14.6	16.1	0.419	0.456	6.7	7.5	3.75	3.95	1.05	0.91*		
						Subject Y						
7–10	13.7	14.9	0.399	0.428	6.1	7.1	3.74	3.67	0.98	0.69	0.66	0.96
7–17	14.3	15.1	0.417	0.438	6.6	7.1	3.72	3.78	1.02	0.63	0.68	1.08
7–19	14.3	15.2	0.420	0.444	6.6*		3.74*			0.65	0.68	1.01
7–21	16.2	16.5	0.481	0.486	7.3	7.8	4.28	4.12	0.96	0.67	0.68	1.05
7–23	15.8	16.4	0.464	0.480	6.9	7.7	4.27	4.10	0.96	0.64	0.71	1.11
7–25	15.6	16.1	0.447	0.468	6.9	7.5	4.09	4.04	0.99	0.72	0.71	0.99
7–27	14.6	15.4	0.418	0.435	6.6	7.1	3.80	3.84	1.01	0.66	0.68	1.03
7–29	14.3	15.1	0.417	0.437	6.5	7.1	3.77	3.78	1.00	0.63	0.66	1.05
Mean	14.9	15.8	0.433	0.459	6.7	7.5	3.92	3.91	1.00	0.79	0.81	1.03†

* Not included in calculating mean values.

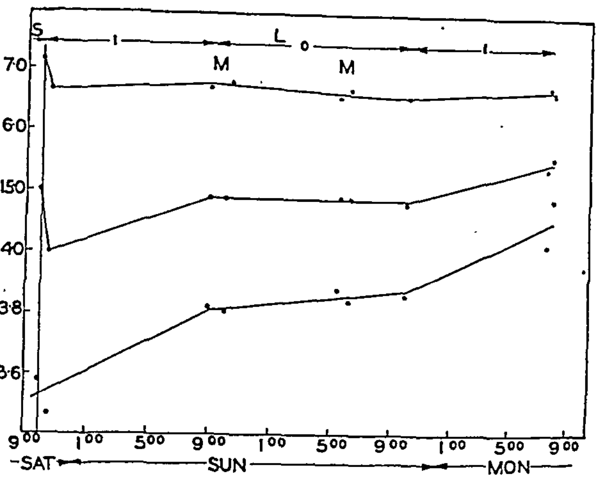
† The increase in quantity of hemoglobin during standing is probably apparent rather than real. From work of others (13), it seems likely that about 3 per cent of the carbon monoxide present in the circulatory system would escape to body tissues during the half hour period.

may be lessened somewhat by the fact that each subject did not begin the two experiments (which were carried out on different days) with exactly the same ratio. The experiments in which a subject was confined to a room maintained at a constant temperature were more satisfactory. Alternate periods of sitting and recumbency caused the ratio of total hemoglobin to total serum protein to

5



6



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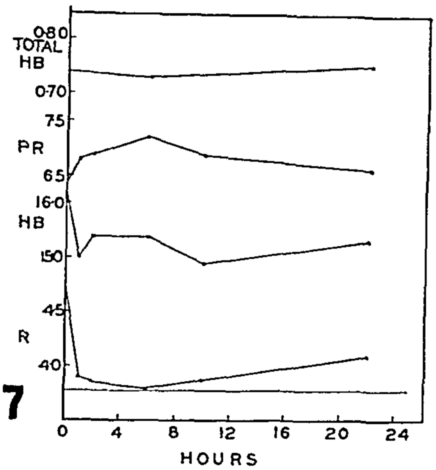

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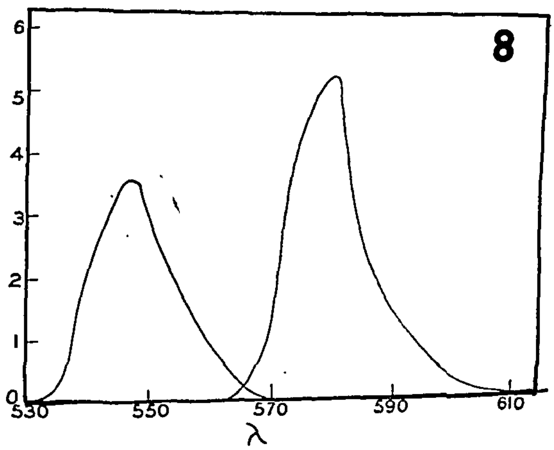

Fig. 5. This figure is similar to figure 4; however each period lasted 12 hours. Total hemoglobin (in kgm.) was determined (by carbon monoxide) at the beginning, middle and end of the experiment. All determinations were made with the subject lying down; for this reason graphs for hemoglobin and protein concentration are not continued through the sitting period (*S*).

Fig. 6. This figure is constructed in the same manner as figure 4. The subject (*R*) lay down throughout the whole period of 34 hours.

Fig. 7. Influence of plasma infusion on protein concentration (*Pr*), hemoglobin concentration (*Hb*), total quantity (in kgm.) of hemoglobin (determined by carbon monoxide) and the ratio (*R*) of total quantity of hemoglobin to total quantity of plasma protein following an infusion of a volume of plasma containing 42 grams' protein. Infusion was complete by the end of the first hour. Samples of blood taken 15–45 minutes later showed the ratio had declined 88 per cent of the theoretical amount. (The level that the ratio should have reached is indicated by a line in the figure.) In three other normal subjects who also received infusion of plasma, the ratios 15–45 minutes after the infusion had declined 90, 68, and 102 per cent respectively of the amount expected theoretically.

Fig. 8. The wave lengths of the light in $m\mu$ are plotted as abscissae and the per cent absolute transmission as ordinates. Since a mercury vapor lamp source is utilized with little background light between λ546 and λ578, most of the energies transmitted by the two filters are at those wave lengths. The curve to the left represents the green, that to the right, the yellow filter. Both represent the combinations used without the inclusion of Kalichrome C.

decrease or increase respectively. Results of some of our experiments are shown in figures 4, 5 and 6. Changes in the ratio occurred irrespective of time of day and were not related to meals. The ratio appeared to increase somewhat more rapidly during sleep than during periods of recumbency in which the subject was awake. Possibly the greater activity during the periods of wakefulness (reading was allowed) was responsible for this difference.

It was found in representative experiments of this study of posture that hemoglobin remained constant in quantity (fig. 5); consequently alteration in quantity of serum protein was responsible for alteration in the ratio of total hemoglobin to total plasma protein.

Plasma infusion. Our procedures estimate with reasonable accuracy the magnitude of alteration of plasma protein resulting from plasma infusion. Data obtained in one experiment are shown in figure 7 and a summary of the pertinent results of three other experiments are described under the heading of this figure.

DISCUSSION. We have utilized extensively throughout this study the easily determinable ratio $\left[\frac{\text{total hemoglobin}}{\text{total plasma protein}}\right]$ as an index of change in quantity of hemoglobin relative to quantity of plasma protein.[9] In all of our experiments of short duration, we have assumed that hemoglobin remained constant in amount and that a change in this ratio signified a change in amount of circulating plasma protein. Determinations of total hemoglobin (by carbon monoxide) in representative experiments justify this assumption in our study.

It is probable that hemoglobin remains very constant in amount for long periods in normal individuals, since only about one per cent of erythrocytes are destroyed and replaced daily (8). In a nearly completed study in which total hemoglobin was determined at short intervals over a period of more than a year, we have found the quantity of hemoglobin to be very constant in each individual[10], except during the summer when circulating hemoglobin is increased in quantity (by 10 to 15 per cent).

The validity of our procedure for estimating changes in amount of circulating protein is also indicated by the fact that we are able to estimate fairly accurately the increase in quantity of circulating protein induced by plasma infusion. However, it is recognized that absolute values for changes in amount of plasma protein are in error to the extent that the ratio $\left[\frac{\text{total hemoglobin}}{\text{total plasma protein}}\right]$ in a sample of blood from an antecubital vein differs from the ratio for the circulatory system as a whole. If there is an excess of plasma over red cells in arteriolar or other small vessels amounting to about ten per cent of the total plasma (9), our estimates of magnitude of change will be in error to that extent.

Effects of environmental temperature upon blood volume have been reported

[9] In some of the studies, plasma protein was determined; in others, serum protein (see legends of tables and figures). Since these differ in quantity by only a small amount, the term plasma protein is used for either to simplify discussion.

[10] Most values are within 5 per cent of corresponding mean values. Since technical errors would cause this degree of variation, it is probable that the quantity of hemoglobin is more constant than is suggested by our data.

previously by others (1, 2, 3). In our experiments in which subjects were exposed to a given environment for about one week, we found that changes in plasma volume accounted entirely for alteration in blood volume. Plasma volume was increased in the heat by simple dilution (as indicated by a lower protein concentration) and by an increase in total amount of protein. In a typical instance blood volume of subject R was estimated to increase on exposure to heat from 4.91 liters (on 12–27) to 5.52 liters (on 1–4), making a total increase of 0.61 liter. These calculations are based upon values from figure 2 for total hemoglobin (0.82 and 0.80 kgm. respectively for the above dates) and values from table 1 for morning hemoglobin concentrations (16.7 and 14.5 grams 100 cc. respectively). It may further be calculated from the above blood volume estimates and from hematocrits of table 1 that plasma volume increased 0.61 liter (from 2.68 to 3.29 liters) and that plasma protein increased in quantity from 193 to 214 grams. The concentration of plasma protein declined from 7.2 to 6.5 grams/100 cc. The reverse changes occurred in the cold.

It is possible that alteration in concentration of plasma protein is only temporary (a few weeks) since in our experiments there seemed to be a tendency for plasma protein concentration to return toward intermediate levels during exposure to the temperature extremes. This interpretation is supported by the fact that plasma protein concentration of men in the tropics (10) is not maintained at levels lower than those considered normal for temperate climates. We have not observed any change in quantity of circulating hemoglobin during these experiments, though this has been observed by others. Perhaps the reason for this discrepancy is individual variability in speed and degree of response to environmental temperature. It is also probable that some of the earlier studies were complicated by seasonal variations in hemoglobin which are of rather large magnitude. However our results appear to be in agreement with those obtained recently (11) in another laboratory.

During the winter experiment, it was found that the ratio $\left[\frac{\text{total hemoglobin}}{\text{total plasma protein}}\right]$ was smaller at night than in the morning by approximately four per cent (table 1). This was tentatively interpreted to mean that extra plasma protein was added to the circulatory system during the day and removed at night, since it seemed unlikely that diurnal changes of this magnitude could occur in hemoglobin. This interpretation was confirmed in subsequent experiments (table 2) which showed that the quantity of hemoglobin (as determined by CO) was approximately the same at night as in the morning.

Further study of diurnal variation in amount of plasma protein indicates that bodily posture is an important causal factor. Plasma protein increases or decreases progressively in amount (according to whether subjects are erect or recumbent, respectively) for as long as we have made observations (12 hrs., sitting; 34 hrs., lying). It seems likely that this phenomenon is related to the decrease in plasma volume which has been reported in studies on the effects of prolonged bed rest (12).

We have not yet determined whether changes in quantity of plasma protein,

which are induced by alteration of environmental temperature or posture, are brought about by storage and liberation or by production and destruction of protein.

SUMMARY

1. Modification of the volume and composition of the blood induced by chronic exposure (several days) to uncomfortably warm and uncomfortably cold environments and by prolonged maintenance (several hours) of the erect or recumbent posture were investigated.

2. The concentrations of plasma protein and hemoglobin decreased on exposure to heat and increased on exposure to cold.

3. Plasma protein increased progressively in amount during the first few days of exposure to heat and decreased somewhat more rapidly on exposure to cold. These changes probably are permanent adaptations to the environment.

4. A measurable change in amount of circulating hemoglobin did not occur during an exposure of a week to either environmental extreme.

5. Plasma protein increased in amount when the upright position was maintained and decreased progressively in the recumbent position. A detectable change in amount of circulating hemoglobin did not occur during these experiments, which lasted several hours.

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APPENDIX

Modifications of the Optical Filters and Photocells Adopted to Improve Stability

M. E. MAXFIELD and H. C. BAZETT

After publication of the original method, instability developed which was associated with the use of later samples of General Electric AH4 mercury vapor lamps, which differed from those obtained earlier. The original filters were designed initially for use with R. C. A. photocells 917 and 919 and had unnecessarily low transmissions for the red end of the spectrum, to which 929 photocells

were insensitive. Stability was found to be decreased by reducing the transmission of the red end of the spectrum and increased by a similar change at the violet end. Probably this instability depended to some extent on a variable degree of contamination of the signal lights by some band of shorter wave length. An additional factor appeared to be the small size of the plate of the 929 photocells, with occasional scatter of light beyond the sensitive margin of the cells. This probably depended on the use of hand-made absorption cells of soft glass which were not completely optically flat. As the result of difficulties imposed by the war, corrections of these sources of error were discovered empirically on the basis of tests for stability, when the lamp used, the voltage on the lamp, and the room temperature were varied.

To improve the mechanical arrangement 929 photocells were replaced by R. C. A. 935 photocells (then in development). These photocells are of larger size than the 929 but have a similar spectral sensitivity. A number of these were obtained and were found to be usable interchangeably except for a single cell which was aberrant and had to be discarded. The absorption cells were replaced also with others of pyrex glass made mechanically and optically flat. One part of the light filter ultimately used was a 2 mm. thick Kalichrome C glass made by Bausch and Lomb. One of these was inserted on each side in the tube connecting the photocell box with the bellows, and the insertion was made approximately air-tight. This prevented the heat from the lamp drawing room air through the photocell boxes and consequently made it easier to maintain dry both these boxes and the electrical equipment within them.

The filter finally used for λ546 was didymium (Corning) 10 mm., Corning 351, 5.9 mm., Wratten 62 cemented between these, and Kalichrome C 2 mm. (Bausch and Lomb) mounted separately at the entrance to the box as already described. That used for λ 578 was the Zeiss A combination but without the additional Jena B. G. 7 previously employed. Instead an additional filter of Corning 348 with a thickness of 4.7 mm. was cemented to the Zeiss A combination. The filter for λ 578 was also reinforced by the Kalichrome C (2 mm.) present in the tube. In the absence of the B. G. 7, the yellow filter allows considerable transmission of a band in the red of wave length above 670 mμ and with a maximum of about 3 per cent transmission at a wave length of 708 mμ. This band is beyond the range of sensitivity of the 935 or 929 photocells. It does not appear necessary to remove it, if these photocells are used.

The transmission values of the filters, kindly determined for us by Dr. D. L. Drabkin, are shown in figure 8. Owing to the higher sensitivity of the photocells to the violet end of the spectrum the maximal energy value of light should be at λ 546 for the green and λ 578 for the yellow filter. The addition of Kalichrome C to these filters should have no appreciable effect on the transmission in the wave lengths used other than some diminution of light by reflection.

In the absence of sources of supply for Zeiss A filters or of Jena glass, a new combination for yellow is required. However, tests of new filter combinations await a convenient time when they will not interfere with other work.

A COMPARISON OF TEN INFUSION FLUIDS IN THE TREATMENT OF MODERATE AND SEVERE HEMORRHAGE IN ANIMALS[1]

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Clinical experience and experimental studies have shown that within certain limits the changes produced by hemorrhage may be reversed if blood volume is restored by infusion of plasma. The present study was planned to evaluate the effectiveness of certain plasma substitutes under conditions of circulatory deficiency usually reversible by plasma therapy.

The plan of study included *1*, response of normal rabbits and dogs to infusions of the fluids under investigation; *2*, response to the fluids given immediately following massive hemorrhage (B.P.—20 mm. Hg), and *3*, response to the fluids given after one hour of hypotension (30 mm. Hg) produced by hemorrhage.

Although the maintenance of a satisfactory blood volume is the primary purpose of a plasma substitute, no one empirical criterion, such as blood volume or survival for a limited time, gives entirely satisfactory evidence for comparing the efficacy of colloidal fluids. An empirical comparison of blood substitutes has been made by several investigators by substituting the fluid under investigation for the volume of blood removed during the first hemorrhage (H_1) and subjecting the same animal to a second hemorrhage (H_2) for the arrival at an H_2 to H_1 ratio (2, 4–7).

In this study response to a second bleeding was considered in addition to survival and changes in blood pressure, hematocrit and plasma protein concentration.

Materials and methods. The following infusion fluids were tested:

(1) Modified human globin (3.3 grams per 100 cc.).[2]
(2) Modified dog globin.
(3) Oxypolygelatin (5.0 grams per 100 cc.).[3]
(4) Human serum albumin (25.0 grams per 100 cc.).[4]
(5) Human serum albumin (5.0 grams per 100 cc.).
(6) Human hemoglobin (7.0 grams per 100 cc.).[5]
(7) Whole blood.

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Pennsylvania.

[2] Smith, Kline and French Laboratories, Lots E2644 and E4444b.

[3] Lot 11381Y

[4] Lederle Laboratories, Inc., Lots 119H341H, K, L, M and 119H329K.

[5] Sharp and Dohme, Lot 1029H.A.

(8) Heparinized pooled plasma (routinely prepared).
(9) Heparinized pooled plasma (especially prepared from heparinized donors).
(10) Physiological saline solution.

Both globin preparations were made under the direction of Dr. Max Strumia. The dog globin was an experimental batch and was not entirely satisfactory according to the chemical criteria established for modified human globin.

Oxypolygelatin was developed to provide fluidity at room temperature and optimum retention in the circulation after infusion (8). The original colloid osmotic pressure was about 58 mm. Hg, but the lot used contained a small amount of gelatinous precipitate which was filtered out immediately before use.

The 25 per cent human serum albumin also contained a macroscopic precipitate in the case of 5 lots. The 5 per cent solution was prepared from the 25 per cent by dilution with physiological saline solution.

The human hemoglobin solution was supplied by the Medical Research Division of Sharp and Dohme.

Blood for plasma pools was obtained by rapid bleeding of lightly anesthetized animals to the point of respiratory distress (2.5 to 5.0 Toronto units of heparin per cc. were added as an anticoagulant in the container into which the blood was drawn). The plasma pools were stored at 4° to 8°C. for twenty-four hours or more.

Blood for autotransfusion and for plasma designated as special plasma was obtained from heparinized animals (100 Toronto units per kilogram of body weight), and 5 to 10 units of heparin per cc. were added after withdrawal. Glass cannulas were used for removal of blood from all donors, and all donors were lightly anesthetized. Such plasma pools were stored at 4° to 8°C. for three to twenty-four hours.

Experimental procedures. *General methods.* Healthy dogs weighing 8.0 to 12.0 kgm. were isolated in individual cages and fed a balanced diet for a week or longer. For sixteen hours prior to an experiment food was withheld but water was available.

All equipment used for blood collections and infusions was chemically cleaned and heat sterilized.

Small blood samples were taken by arterial needle puncture and kept fluid by additions of 0.25 mgm. of heparin per cc. In animals with prolonged hypotension a needle was left indwelling in the jugular vein for this purpose.

Hematocrit determinations were made in Wintrobe tubes centrifuged for one hour at 2,500 R.P.M. (radius to bottom of tube 14.0 cm.). The protein content of plasma was measured with the falling drop method of Barbour and Hamilton (1) using the formula of Weech, Reeves and Goettsch (9) for conversion of specific gravity to protein content. In the presence of injected hemoglobin the specific gravity determinations were unsatisfactory and are not included in this study.

All fluids infused into dogs following hemorrhage were given at a temperature of 28° to 32°C.

Administration of infusion fluids to normal dogs and rabbits (not subjected to

hemorrhage) for observations of possible untoward effects. The material to be tested was infused in a volume equal to 2 per cent of body weight by means of a needle introduced into a jugular vein in dogs. In rabbits a needle was introduced into an ear vein. The rate of infusion was 4.0 cc. per kgm. per minute except in the case of globin when slower infusion rates were often employed. In dogs a femoral artery was cannulated under local anesthesia in order to record blood pressure.

First hemorrhage procedure (immediate replacement of the blood withdrawn by the infusion fluid to be tested). The steps in this procedure were as follows:

1. Anesthesia. A slow intravenous injection of a maximum of 24.0 mgm. per kgm. of pentobarbital sodium was given as the initial dose.

2. Cannulation. A femoral artery was cannulated for determining mean arterial pressure and for withdrawing blood. A sterile 10 per cent sodium citrate solution was used in connecting the artery to the mercury manometer. A 15 gauge needle was inserted into the jugular vein and left indwelling for infusions. When not in use it was kept filled with heparinized saline.

3. First hemorrhage. About thirty minutes after anesthetization the dogs were bled at the rate of 4.0 cc. per kilogram per minute to a blood pressure end-point of 20 mm. Hg. Blood collections were made in 200 cc. bleeding bottle units. Delays of a few seconds each were necessary for changing the bleeding tubes. During the change the cannula was flushed and emptied of sterile citrate solution by a syringe system. Blood lost in making bleeding unit changes was noted in the total withdrawal volume. The rate usually slowed fairly markedly during the last minute or so as the end-point was approached. An end-point near 30 mm. Hg was necessary in three animals due to temporary respiratory failure.

4. First infusion. Within one minute of the completion of the first bleeding an infusion of the material to be tested was started. The amount and rate of this infusion corresponded to the amount of blood removed and the rate of its withdrawal.

5. This first infusion was followed by a three hour period of observation. Small supplementary doses of pentobarbital sodium were given to maintain light anesthesia when required.

6. Second bleeding. The animals were bled at the same rate (4.0 cc. per kgm. per minute) and to the same end-point (B.P. = 20 mm. Hg) as before and the volume of blood withdrawn carefully measured for comparison with the first bleeding.

7. Immediately after the second bleeding was completed a second infusion was begun. This infusion was composed of the erythrocytes removed at the first bleeding suspended in the same kind of fluid used for the first infusion. The volume replaced at the second infusion was equal to the volume of the first hemorrhage. The animals remained in a supine position until one hour following the second bleeding. After ligation of the femoral artery the wound was irrigated with copious amounts of sterile saline solution and closed by suture.

A modification of Procedure I with an end-point of 30 mm. Hg and infusion

rates of 0.5 to 1.0 cc. per kgm. per minute was used in two experiments with globin.

Second hemorrhage procedure (an hour of hypotension before replacement of the blood withdrawn by the infusion fluid to be tested). This procedure differed from the first hemorrhage procedure as follows:

The first bleeding was terminated when the blood pressure fell to near 30 mm. Hg. By repeated careful small blood withdrawals or injections an average mean pressure level of 30 mm. Hg was maintained for one hour. A small portion of blood from the initial bleeding was kept at room temperature for these injections (using 5 Toronto units of heparin per cc.).

At the end of the hour of hypotension an infusion of the material to be tested in a volume equal to the first bleeding[6] was given, but the rate was only 2.0 cc. per kgm. per minute. The second bleeding was begun three hours after the first infusion.

In the case of 25 per cent albumin both rate and volume were reduced to one-fifth that used for the other fluids, and the erythrocyte suspension was made with 5 per cent albumin solution for the second infusion.

Methods of analyzing the data. When values for the hematocrit and plasma protein concentration obtained postinfusion and the volume of the second bleeding were expressed as per cent of a control observation made in the same animal, the results gave clear patterns both in Procedure I and Procedure II.

The following were chosen as the control values:

(1) Serum protein concentration before anesthesia.

(2) Hematocrit before the first infusion.

(3) Blood pressure level between anesthesia and first hemorrhage.

Bleeding volume index was expressed as the ratio of the second hemorrhage to the first in Procedure I.

In Procedure II the volume of first bleeding to the time the end-point was first reached was used as the denominator. This same figure was used to determine the volume of the first infusion.

RESULTS. *Observations of the effect of various infusions in normal animals (not subjected to hemorrhage).* (1) Rabbits. The fluids under investigation produced no obvious unfavorable reactions.

(2) Dogs. Human and dog globin (at 4.0 cc. per kgm. per min.) caused untoward reactions which included respiratory distress, vomiting, flushing of the skin and urticaria. With infusion rates of 2.0 to 4.0 cc. per kgm. per minute the blood pressure fell to levels 52 to 75 per cent below the control level, but infusion rates up to 1.0 cc. per kgm. per minute produced insignificant pressure changes.

Human serum albumin (5 per cent), oxypolygelatin, and heparinized dog plasma produced no obvious untoward reactions during infusion. However, following infusion of standard heparinized plasma (stored twenty-four hours or more) dogs developed cutaneous wheals and occasionally facial edema.[7]

Special plasma seldom caused these cutaneous reactions.

[6] The first bleeding averaged 7.0 cc. per kgm. less than the total volume of blood removed.

[7] Further study of this reaction and the methods of avoiding it are in progress.

The plasma of dogs which had received human serum albumin always developed an icteric tint by one and one-half hours after infusion though tests for bilirubin gave values which did not exceed 0.5 mgm. per 100 cc.

Sedimentation rates were accelerated markedly by oxypolygelatin and slightly by heparinized plasma. They were decreased by globin, hemoglobin, 5 per cent albumin and physiological saline solution.

Results with the first bleeding procedure. The average volume of blood removed to reduce the blood pressure from the average initial level of 148 mm. Hg to the

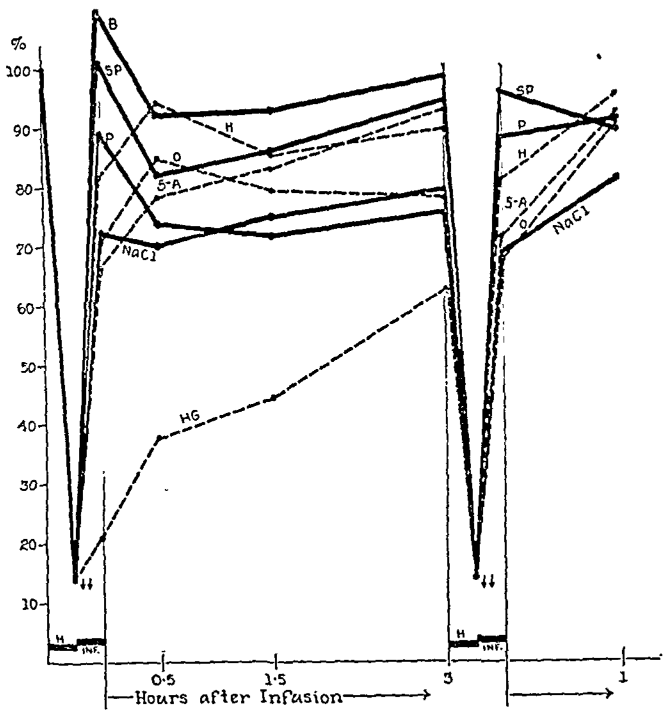

Fig. 1. Mean blood pressure changes for the several groups of animals subjected to bleeding to a blood pressure end-point of 20 mm. Hg and immediately infused (Procedure I). Each curve represents the animals infused with one preparation. *B* = whole blood (autotransfusion); *P* = plasma; *SP* = special plasma; *H* = hemoglobin; *O* = oxypolygelatin; 5-*A* = 5 per cent albumin; *NaCl* = physiological saline solution; *HG* = human globin.

selected end-point of 20 mm. Hg was 51.7 cc. per kgm. No animals died before the first infusion. All of the infusion fluids except rapidly infused globin restored and maintained the blood pressure satisfactorily though the promptness of the response varied (fig. 1).

The bleeding volume index, which we regarded as the best single criterion for evaluating the infusion fluids, was highest for whole blood, 95 per cent; and human serum albumin, 88 per cent. At the other extreme was modified human globin, 0 and 43 per cent (51.7 per cent with a modified procedure) and dog globin, 0 and 13 per cent (34 per cent with a modified procedure). In the intermediate range was saline, 48 per cent; standard plasma, 65 per cent; hemoglobin, 73 per cent;

oxypolygelatin, 79 per cent and special plasma (from heparinized donors), 81 per cent.

We understand that further improvements in the manufacture of modified human globin have been made since experiments were done.

Hematocrit and plasma protein concentration change. The hematocrit and plasma protein changes are shown graphically in figures 2 and 3 respectively. Each line represents the average for all dogs receiving one infusion fluid.

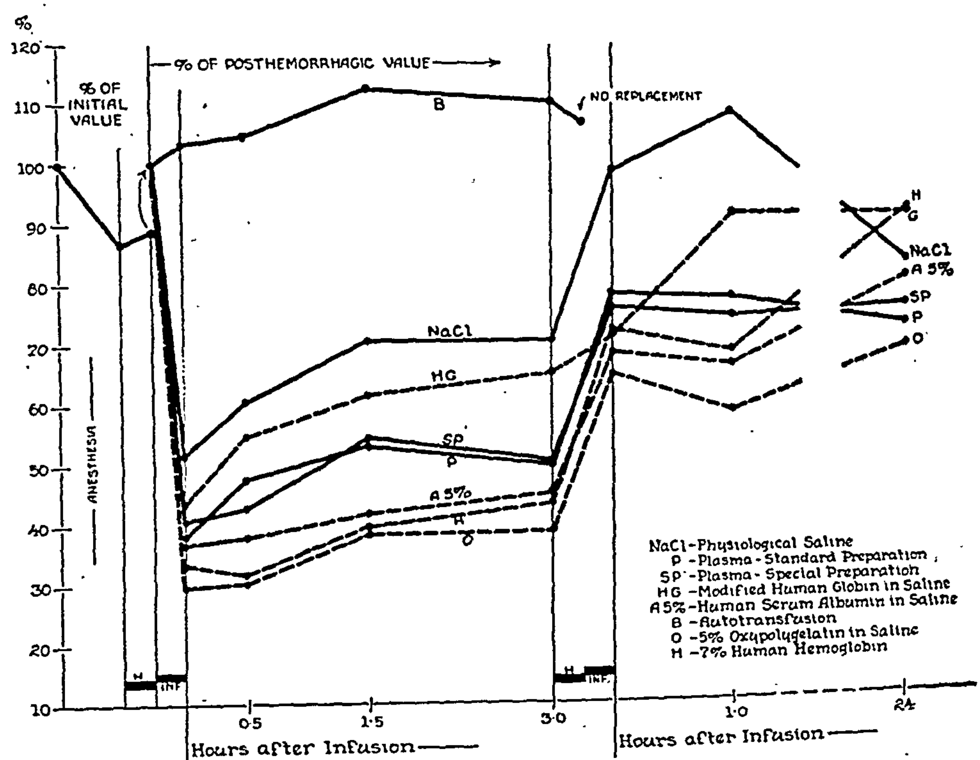

Fig. 2. Mean hematocrit changes in Procedure I following infusions of various fluids. Each curve represents the means of the values obtained in the animals receiving the particular fluid indicated. Prior to the end of the hemorrhage the values are given in per cent of the initial reading. Subsequent values are given in per cent of the value obtained at the end of the hemorrhage. B = whole blood (autotransfusion); P = plasma; SP = special plasma; A 5 per cent = 5 per cent albumin; HG = human globin; O = oxypolygelatin; $NaCl$ = physiological saline solution.

The individual curves for protein concentration showed very little deviation from the average. For instance, the values at one and one-half hours postinfusion ranged from 65 to 69 per cent of the initial value in five dogs infused with physiological saline solution and from 69 to 78 per cent in six dogs infused with oxypolygelatin. The individual curves for hematocrit changes were also remarkably uniform following infusions of physiological saline solution, but fairly large individual variations were present following infusions of oxypolygelatin. This may have been related to an effect of this material on the distribution of red blood cells.

Survivals. Physiological saline solution permitted indefinite survival following Procedure I. No replacements were made in the autotransfusion group after the second hemorrhage, and death occurred in about thirty minutes. Two of three animals dying of respiratory infections in the week following the experiment exhibited rapid erythrocyte sedimentation as evidence of probable morbidity prior to bleeding. Table 1 lists the survivals during the first week for Procedure I.

Results with Procedure II (infusion after 1 hr. of hypotension). With this procedure it was observed that dilution of the circulating plasma occurred during the period of hypotension but was largely complete in thirty minutes. The average

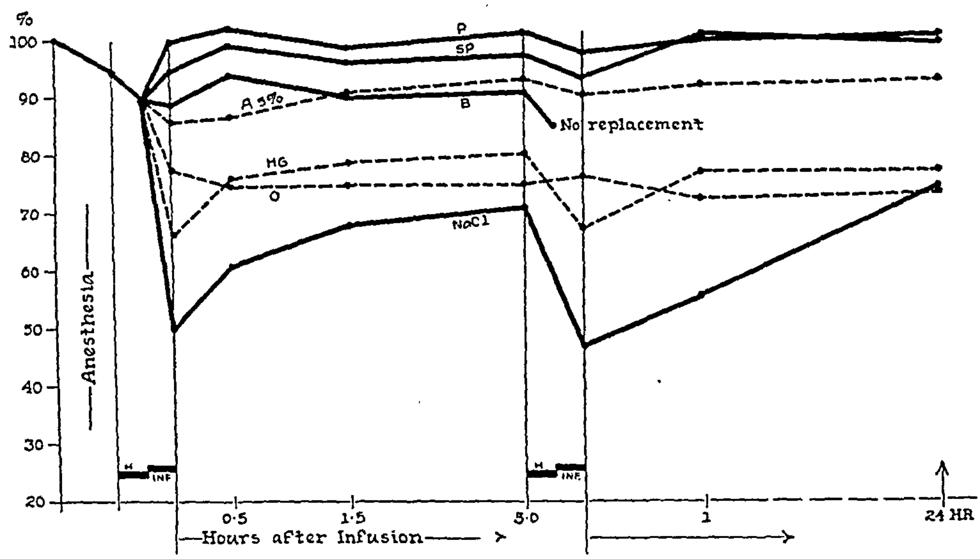

Fig. 3. Mean changes in plasma protein concentration in animals subjected to hemorrhage (to B.P. of 20 mm. Hg) and immediate infusion. Each curve represents the results obtained with a single fluid.

initial bleeding was 42.4 cc. per kgm., but the additional bleeding required to maintain the hypotension for one hour averaged 7.2 cc. per kgm. more. One dog died during the hypotensive period. Data on all of the other dogs have been included in table 2.

Globin, whole blood and standard plasma were not employed with Procedure II, but all of the other materials produced a blood pressure rise. The levels ranged from 64 to 117 mm. Hg immediately following infusion and were similar for all groups. One animal receiving hemoglobin died in the course of the infusion after the B.P. had reached 60.

The pressure was maintained in every animal in the groups infused with 5 per cent albumin and special plasma.

The second bleeding was not carried out either because the dog had died or because the B.P. had already declined to the 30 mm. Hg end-point in 60 per cent of the animals receiving physiological saline solution, 20 per cent of the group receiving 25 per cent albumin and 50 per cent of those receiving hemoglobin solu-

TABLE 1

Experimental procedure I—immediate infusion

INFUSION FLUID	EXP. NO.	INITIAL BLEEDING	SECOND BLEEDING		HEMATOCRIT (VOL. %)			SURVIVAL, 7 DAY PERIOD
			cc./kgm.	B.V.†	Preinfusion	Postinfusion 2 min.	Postinfusion 3 hr.	
		cc./kgm.		*Per cent*				
Autogenous blood	B1	53.6**	50.6	94.4	36.6	35.4	42.5	#
	B2	40.5**	42.3	104.0	44.5	48.2	49.0	#
	B3	42.2**	36.2	85.6	38.3	40.2	42.2	#
Physiological saline solution	S1	60.0	30.6	50.1	45.2	22.2	32.2	+
	S2	53.4	26.1	48.8	42.5	24.1	32.6	+
	S3	53.6	22.6	42.0	38.2	20.0	25.1	+
	S4	50.7	23.0	45.2	38.2	19.0	26.4	+
	S5	49.0	26.0	53.5	37.0	18.7	25.5	+
Heparinized pooled plasma	P1	53.3	32.8	61.4	40.0	14.2	17.7	+
	P2	48.8	31.1	63.6	46.0	13.1	19.5	+
	P3	47.0	29.1	62.0	42.4	19.6	23.3	+
	P4	44.2	31.8	72.0	41.5	16.7	23.1	+
	P5	48.0	32.4	67.7	42.9	17.8	22.8	+
"Special" plasma	SP1	50.5	40.7	80.6	44.0	17.4	25.2	Died 5th day—Pneumonia
	SP2	47.8	38.9	81.3	26.4	11.1	14.4	+ Severe anemia
5% human serum albumin	A1	57.4	44.4	77.3	39.7	16.9	23.0	Died 6th day—Pneumonia
	A2	42.5	40.3	94.8	43.1	18.5	23.6	Died 3rd day—Purulent empyema
	A3	53.7	50.0	93.2	47.9	16.3	19.3	+
	A4	47.2	37.3	79.0	41.3	11.5	13.1	+
	A5	57.6	54.8	95.2	40.4	14.3	15.4	#
7% human hemoglobin	H1	52.0	41.6	80.6	38.0	11.8	19.0	+
	H2	54.7	29.7	54.4	42.2	13.6	19.1	+
	H3	42.8	35.7	83.4	35.0	12.7	12.6	+
5% oxypolygelatin	O1	54.6	38.6	70.7	42.3	9.8	21.4	+
	O2	60.8	44.1	72.5	40.0	7.5	11.2	+
	O3	50.3	40.3	80.1	36.7	9.2	12.4	+
	O4	45.2	37.8	83.8	42.1	17.4	20.5	Died at end of 2nd infusion
	O5	61.3	49.1	80.1	46.3	15.8	13.5	+
	O6	51.7	43.7	84.7	44.7	15.9	19.3	#
Modified human globin	HG1	45.0	19.4	42.8		17.4	27.3	Died during second infusion
	HG2	47.4	0	0				Died during first infusion
	HG3*	51.4	26.8	51.7	45.2	19.6	29.4	+
Modified dog globin	DG1	52.0	0	0				Died during first infusion
	DG2*	46.0	15.8	34.5	36.9	21.7	27.3	+
	DG3	56.7	7.3	12.8	42.8	23.9	32.1	Died during second infusion

* A modification of Procedure I was used.

** Volume of blood returned; 3.6 to 5.4 cc./kgm. of blood was lost by manipulations used to insure satisfactory fluidity

No fluid replacement following the second hemorrhage. Death occurred within 30 minutes after the second hemorrhage.

† The second bleeding volume expressed as the per cent of the volume replaced following the first hemorrhage represents the "Bleeding Volume."

tion. Consequently the following comparison of bleeding volume indices entails a selection of experiments:

With special plasma the average bleeding volume was 66 per cent, with 5 per

cent albumin, 59 per cent; and with 25 per cent albumin (in those that lived), 57 per cent. Oxypolygelatin gave an average of 48 per cent; physiological saline solution, 28 per cent; and the hemoglobin, 19 per cent. In the case of the oxypolygelatin there was a rather wide range, but it was well correlated with the hemodilution just before the second hemorrhage.

TABLE 2

Experimental procedure II—delayed infusion

INFUSION FLUID	EXPER. NO.	INITIAL BLOOD LOSS	FINAL NET BLOOD LOSS	HEMATOCRIT (VOL.%)			B.P. AT 3 HOURS POSTINFUSION	SECOND BLEEDING		SURVIVAL, 24 HOUR PERIOD
				Preinfusion	Postinfusion 2 min.	Postinfusion 3 hr.		cc./kgm.	B.V.	
		cc./kgm.	cc./kgm.				mm. Hg			
Physiological saline solution	2-S1	51.0	54.6	41.7	24.5	37.6	114	17.7	34.5	Died at end of 2nd infusion
	2-S2	40.0	50.3	45.7	27.9			0	0	#
	2-S3	48.2	52.9	37.7	20.1		31	0	0	0**
	2-S4	42.8	55.9	33.0	22.3			0	0	†
	2-S5	32.8	43.5	32.5	23.9	30.8	95	7.4	22.6	+
Special plasma	2-SP1	40.9	50.0*	39.4	20.9	25.3	97	30.2	72.7	Sacrificed for tissues
	2-SP2	47.1	52.6*	39.9	17.1	23.3	82	28.4	60.2	+
	2-SP3	40.5	45.0	42.0	21.0	24.3	96	26.4	65.1	+
	2-SP4	41.1	51.2*	41.3	20.3	21.4	83	26.8	65.3	+
5% human serum albumin	2-A1	32.4	41.9	51.3	23.6	28.6	100	20.6	63.0	+
	2-A2	47.1	48.6*	36.1	15.9	23.4	115	24.7	53.2	+
	2-A3	42.8	52.9	27.4	12.4	15.3	108	25.6	59.7	+
25% human serum albumin	C-A1	49.0	53.8	37.0	25.4	23.1	96	27.9	56.8	+
	C-A2	39.0	46.3	31.7	22.4	Dead		0	0	0
	C-A3	47.7	53.2	35.8	23.7	21.6	112	27.8	58.1	+
	C-A4	44.9	52.4	37.7	21.5	18.8	103	25.7	57.3	+
	C-A5	48.7	56.0	30.8	18.3	16.9	109	27.3	56.3	+
5% oxypolygelatin	2-O1	46.7	48.6	35.7	15.6	20.7	83	22.6	48.0	+
	2-O2	41.7	35.5*	42.8	20.5	31.0	45	10.1	24.0	Died 1 hour after 2nd infusion
	2-O3	42.8	60.4	36.0	15.8	18.2	77	24.2	56.0	Died at end of 2nd infusion
	2-O4	43.6	57.8	39.4	15.1	17.5	96	28.6	65.6	+
7% human hemoglobin	2-H1	30.0	33.9*	48.4	29.3	48.4	43	5.7	19.0	Died 3 hours after 2nd infusion
	2-H2	44.2	39.4*	54.6	Dead			0	0	0

* Small reinfusions of blood required during last half of hypotensive period to combat persistent tendency of blood pressure to decline below 30 mm. Hg.

** Second infusion withheld. Animal lived 1.5 hours with B.P. below 30 mm. Hg

\# After a second period of spontaneous hypotension below 30 mm. Hg the animal was given 2.0 cc./kgm. of 25 per cent albumin. Survived.

† Resuscitated after the blood pressure declined below 20 mm. Hg by artificial respiration and the infusion of erythrocytes in physiological saline solution. Survived.

When the data were analyzed further, we found that of seven dogs which required frequent small transfusions to maintain the B.P. at 30 mm. Hg during the latter part of the hour of hypotension three were in the group receiving special plasma, and one received 5 per cent albumin. That tissue damage was marked in this group is suggested by the occurrence of bloody stools in six of the seven. Thus the special plasma group seems to have been weighted with less resistant

animals. On the other hand, the reactions of the five animals in the physiological saline group during the hour of hypotension suggested that they were superior risks. Thus there is reason to believe that the results underestimate rather than over-estimate the difference in the effectiveness of the special plasma and the saline.

Changes in hematocrit and plasma protein concentration. Curves showing the change in hematocrit and in protein concentrations for the various groups are shown in figures 4 and 5 respectively.

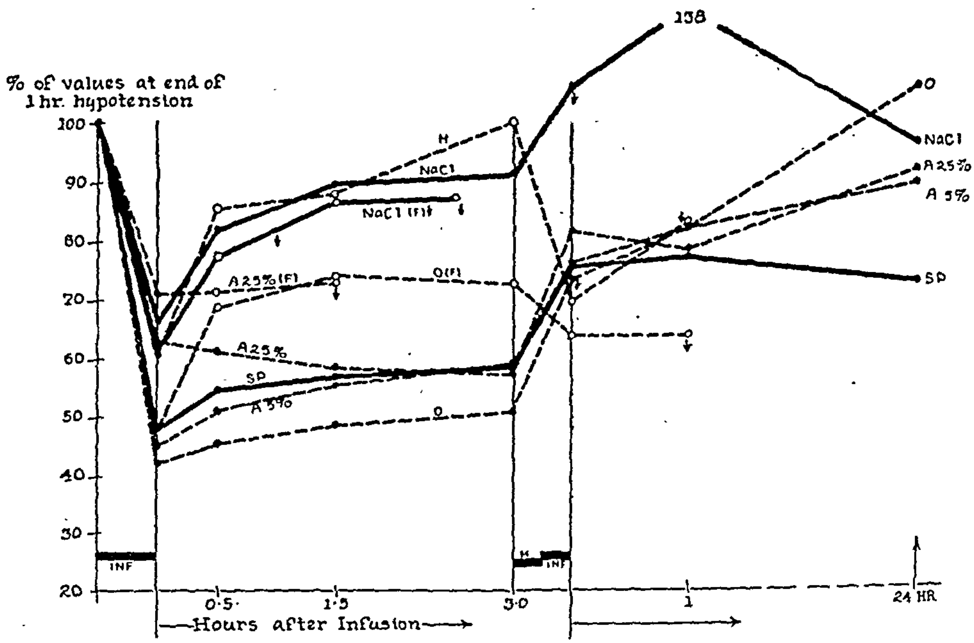

Fig. 4. Mean hematocrit changes obtained with various infusion fluids following an hour of hypotension (B.P.—30 mm. Hg) (Procedure II). Where some of the animals failed in the course of the experiments they are represented by a separate curve from those that did not fail. Such curves are designated *F*, and the arrows indicate the points at which individual animals either had died or the blood pressure had fallen to 30 mm. Hg. *H* = hemoglobin solution; *NaCl* = physiological saline solution; *A* 25 per cent = 25 per cent albumin; *O* = oxypolygelatin; *SP* = special plasma; *A* 5 = 5 per cent albumin.

Each group in which failures occurred during the three hour period is represented by two curves, one for the animals in which the blood pressure failed and one for those animals in which it was maintained.

Survival. The distribution of circulatory failures preceding or following the second hemorrhage is given for the infusion fluid groups in table 2. None of the remaining animals appeared to be in shock at the twenty-four hour interval, but indefinite survival was poor. Most of the animals died within five days to three weeks, and respiratory infection was either a causal or a contributory factor in practically all cases.

Other observations and results. A moderate fall of blood pressure accompanied wheals and facial edema when these appeared following infusions of plasma. With

Procedure I two deaths occurred during the first infusion with globin, and the other two animals receiving this material died during the second infusion. When the infusion rate was reduced to from 0.4 to 1.0 cc. per kilogram per minute, a better blood pressure recovery was obtained with globin.

A decreased tolerance for Nembutal was observed following infusion of oxypolygelatin. Marked pseudo-agglutination occurred with oxypolygelatin making infusion of red cell suspensions difficult technically. Three of nine animals receiving it failed to show blood pressure recovery after the second infusion. Infusions of hemoglobin solutions caused secretion of hemoglobin in urine, saliva and tears, and also excessive diuresis within twenty-four hours.

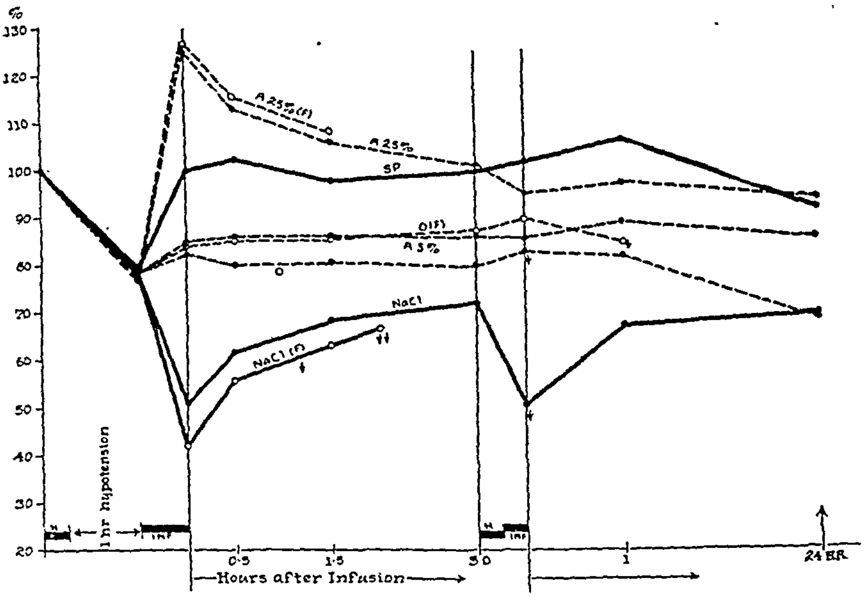

Fig. 5. Mean changes in plasma protein concentration for animals subjected to an hour of hypotension before infusion. Separate curves have been drawn for those animals in each group that failed in the course of the experiments.

Discussion. The clinical observations and the data obtained in this study indicated that globin, standard plasma, oxypolygelatin, and hemoglobin had individual disadvantages which counteracted *in vivo* in varying degrees the advantages conferred by their osmotic activity. It was possible in the experiments with immediate infusion (Procedure I) to show that the globin solutions were unsuitable since the results were inferior to those obtained with physiological saline solution. By this same procedure certain disadvantages of heparinized plasma prepared by a method previously considered acceptable were revealed. The efficacy of heparinized plasma was, however, increased by certain modifications in the method of preparation. The resulting product was designated as "special plasma." It was difficult to evaluate the response to hemoglobin and to oxypolygelatin by the experiments with immediate reinfusion since hemodilution was superior to and the bleeding volumes similar to those obtained with special plasma. The limitations and defects of these two plasma substitutes became evi-

dent in the experiments in which infusion was delayed for one hour (Procedure II). Five per cent albumin gave results comparable to special plasma under the conditions of this procedure, but less satisfactory experience with 25 per cent albumin indicated that fluid in addition to the albumin itself had to be supplied to insure optimum conditions for animal survival.

Our observations emphasized the importance of determining the efficacy of physiological saline solution, the usual diluent for colloids, and indicate the caution that should be observed in the preparation of animal plasma to be used in a study of blood substitutes.

SUMMARY AND CONCLUSION

The effectiveness of ten infusion fluids (human globin, dog globin, whole blood, standard plasma, special plasma, 5 per cent albumin, 25 per cent human albumin, oxypolygelatin, human hemoglobin, and physiological saline solution) in restoring the circulation of dogs subjected to severe hemorrhage was compared.

Two methods were used in producing the hemorrhage. In the first blood pressure was lowered to 20 mm. Hg (Procedure I) by a hemorrhage averaging 51.7 cc. per kgm. and the replacement started immediately. In the second blood pressure was lowered to 30 mm. Hg (Procedure II) and maintained at that level for one hour (average total blood loss, 49.4 cc. per kgm.). In both procedures the dogs were subjected to a test bleeding three hours after infusion.

The human and dog globin gave less satisfactory responses than physiological saline solution with Procedure I and were not studied further. This procedure showed that dog plasma prepared by the usual routine was inferior to plasma obtained from heparinized donors. Accordingly only the latter was tested in Procedure II.

All of the other materials except whole blood were tested in Procedure II, which was a much more severe test and probably more nearly comparable to clinical conditions. Physiological saline solution and hemoglobin solution were definitely inadequate by this test, and oxypolygelatin and 25 per cent albumin were less suitable than especially prepared plasma and 5 per cent albumin.

Unless special precautions were taken to minimize incipient coagulation and to exclude microscopic particulate matter, dog plasma frequently produced subcutaneous edema accompanied by a fall in blood pressure a short time after infusion.

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THE EFFECT OF PULMONARY VASCULAR CONGESTION ON THE DISTENSIBILITY OF THE LUNGS[1]

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Pulmonary congestion operates in various ways to bring about dyspnea. Mechanically, one of the most important of these factors is the change in the distensibility of the lungs. When the pulmonary vessels are engorged with blood they become more rigid, acting like a hose turgid with water under pressure (1). It is readily seen that this is a contributing factor in the increased respiratory effort required in congestive heart failure. This diminished distensibility also contributes to a diminution in vital capacity, and to the formation of intrapleural transudate.

However, only indirect evidence is available to demonstate a diminution in pulmonary distensibility in congestion of the lungs. Drinker, Peabody and Blumgart (2) showed that compression of the pulmonary veins in anesthetized cats diminishes pulmonary ventilation when a constant alternating flow of air into the lungs is maintained. By recording the simultaneous tracings of the tidal air and the intrapleural pressure in patients in congestive failure, Christie and Meakins (3) were able to demonstate, in an indirect fashion, a marked decrease in the distensibility of the lung. Otis, Rahn and Fenn (4) measured the distensibility of the lung in patients indirectly by recording simultaneous venous and intrapulmonary pressures, but they did not apply this method to patients with pulmonary congestion.

We proposed to demonstrate directly the effect of congestion of the pulmonary vessels on lung distensibility, excluding many of the factors which complicated earlier studies. Our studies were carried out 1, with fresh lung preparations obtained from dogs, and 2, in the intact animal.

In the isolated preparation the lungs, with heart attached, were removed from heparinized dogs.[2] A cannula was tied into the pulmonary artery, and a second one was forced through a slit in the left ventricle and tied at the mitral orifice to drain blood from the left atrium and thus from all the pulmonary veins. The rest of the heart was cut away. The trachea was then cannulated, and the entire preparation suspended by the trachea within a special chamber. The cannulae to the pulmonary artery, the left atrium, and to the trachea were passed through to the outside of the chamber. The chamber was sealed. With this preparation it was possible to inject a known quantity of blood into the pulmonary artery and to siphon it off, if desired, through the pulmonary veins.

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[2] Liquaemin was generously supplied by Roche-Organon, Inc.

An apparatus was then designed which made possible the injection of known volumes of air into the lung via the tracheal cannula, and the determination of the pressure inside the trachea (P_1). The "intrathoracic" pressure (P_2) in the sealed chamber in which the lung was contained could be read from a second manometer. The assembly is illustrated in figure 1.

The entire system was brought into equilibrium with atmospheric pressure at the beginning of the experiment. The intratracheal and "intrathoracic" pressures were recorded as progressively increasing volumes of air were injected into the lungs.

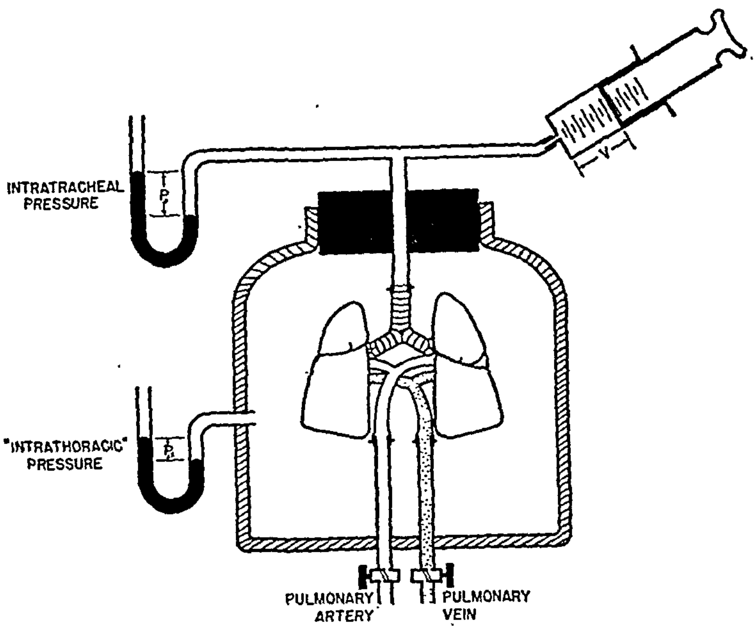

Fig. 1. Diagram of assembly used in measuring volume of air injected intratracheally and the resulting intratracheal, P_1, and intraplethysmographic, P_2, pressures. The method of injecting a measured quantity of air, V, into the lung by means of a syringe is shown diagrammatically. In the experiments reported in the text, water displacement was actually used to measure the volume of air injected. This was accomplished by inserting a pair of water bottles between the syringe and the lungs.

The distensibility of the lung was calculated from plots obtained by comparing the relation between the intratracheal (P_1) and the amount of air (V) injected into the lungs via the trachea, and also by comparing the relationship between the intratracheal (P_1) and "intrathoracic" (P_2) pressures. This estimate of lung distensibility did not change with successive trials in the same lungs. These distensibility determinations were then repeated after the injection of fixed amounts of heparinized filtered blood from the same animal into the pulmonary artery while the outflow cannula from the left atrium was occluded. Definite reduction in distensibility of the lung as indicated in the slope of the curves was produced by the injection of blood, and these changes varied with the amount of blood injected. These reductions in distensibility were reversed when the blood

was siphoned from the lungs via the pulmonary veins. This was demonstrated in 15 preparations.

That the injection of blood into the pulmonary circulation caused a decrease in the distensibility of the lung proportional to the amount of blood introduced, was shown also by a decrease in the slope of the pulmonary volume-intratracheal pressure curve. When almost all the blood injected was siphoned off this curve was also seen to return towards its original location, thus demonstrating clearly that the effect was due to intravascular blood which could be siphoned off, and not to an intra-alveolar transudate (pulmonary edema) which increased slightly during the course of the experiment (fig. 2).

Usually some of the injected blood could not be siphoned off; a certain quantity was always retained in the lungs which could not be recovered.

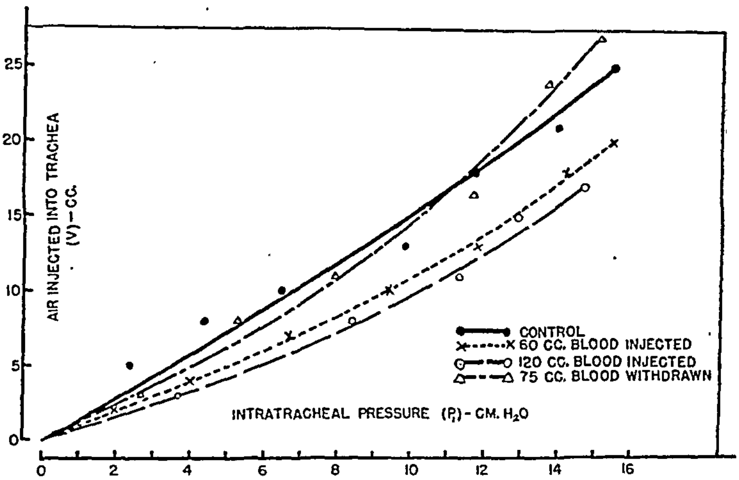

Fig. 2. The effect of pulmonary congestion on the distensibility as shown by volume /intratracheal pressure curves. Blood was injected into the pulmonary artery and siphoned from the pulmonary veins. A decrease in distensibility of the lung is shown by a decrease in the slope of the curve

The discrepancy between the rate of pressure rise in the lung (P_1) and in the closed chamber in which the lung was contained (P_2) caused by the injection of blood is also affected by changes in the distensibility of the lung and is not due just to the effect of the volume changes of the chamber due to the volume of injected blood (fig. 3). This was shown by an experiment in which a rubber balloon was substituted for the lung and fluid added to the chamber, outside of the balloon. The succession of pressure changes within and outside the balloon showed only a negligible deviation from those obtained before the instillation of fluid in the chamber.

The degree of hysteresis in the lungs was determined, and while definitely present, did not affect the slope of the curves and hence the results of the experiments.

A second series of experiments was performed in intact dogs. The dogs were anesthetized with sodium pentobarbital intravenously (25 mgm./kilo). Artificial

respiration was administered through a tracheal cannula. Sufficient curare was given intravenously to prevent spontaneous movements of the thoracic or diaphragmatic musculature even when the artificial respiration was temporarily interrupted. The anterior portion of the thoracic cage was removed. A manometer was connected to the tube joining the trachea to the artificial respiration apparatus. It was then possible to clamp the tube leading to the artificial respiration machine, open the trachea to the atmosphere briefly, then inject given quantities of air into the lung (as in the isolated preparation experiments) and measure the changes in intratracheal pressure produced. In this way a volume

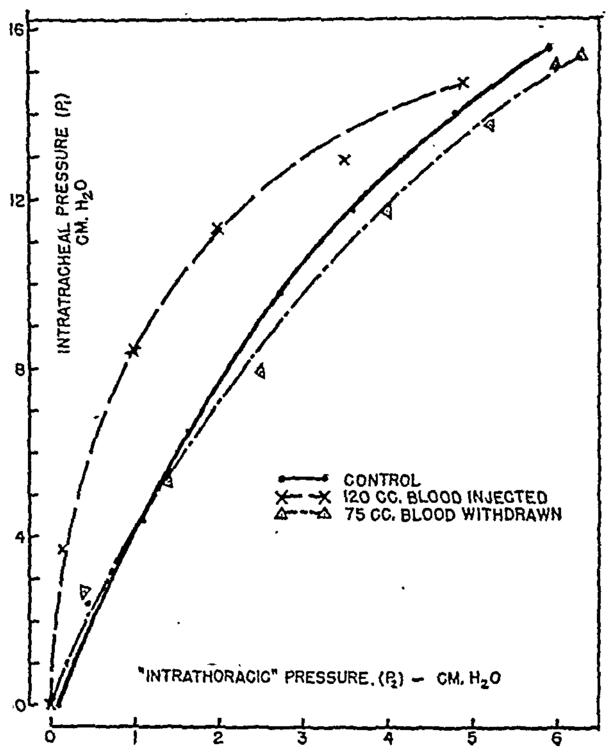

Fig. 3. The effect of pulmonary congestion on the distensibility of the isolated lung as shown in intratracheal/"intrathoracic" pressure curves. Blood was injected into the pulmonary artery and siphoned from the pulmonary veins. A decrease in distensibility of the lung is shown by an increase in the slope of the curve.

pressure curve could be constructed similar to those in the isolated preparation experiments. The series of recordings necessary for this curve, which reflected the degree of distensibility of the lung, could be obtained within 1 minute.

An attempt was then made to produce congestion of the lung in the living animal, and to determine its effect on the distensibility of the lung This was accomplished by placing a rubber tipped clamp on 2 or 3 of the 4 pulmonary veins at their entrance to the left atrium. By obstructing the return of blood from the pulmonary veins this clamp acted much like a very severe mitral stenosis and produced congestion in the lung. Volume-pressure curves obtained when the pulmonary veins were occluded clearly showed a decrease in distensibility of the

lung (fig. 4). On removal of the clamp, distensibility returned to normal within 5 minutes.

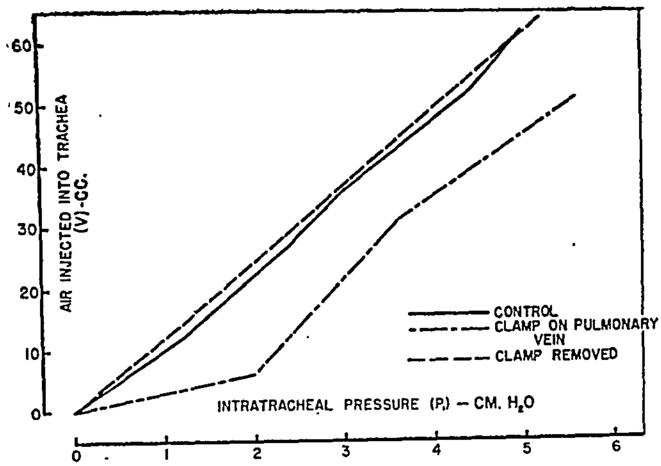

Fig. 4. The effect of pulmonary congestion on the distensibility of the lung in the living dog, as show in volume/intratracheal pressure curves. Congestion of the lungs was produced by compression of the pulmonary veins. A decrease in distensibility is shown by a decrease in the slope of the curve.

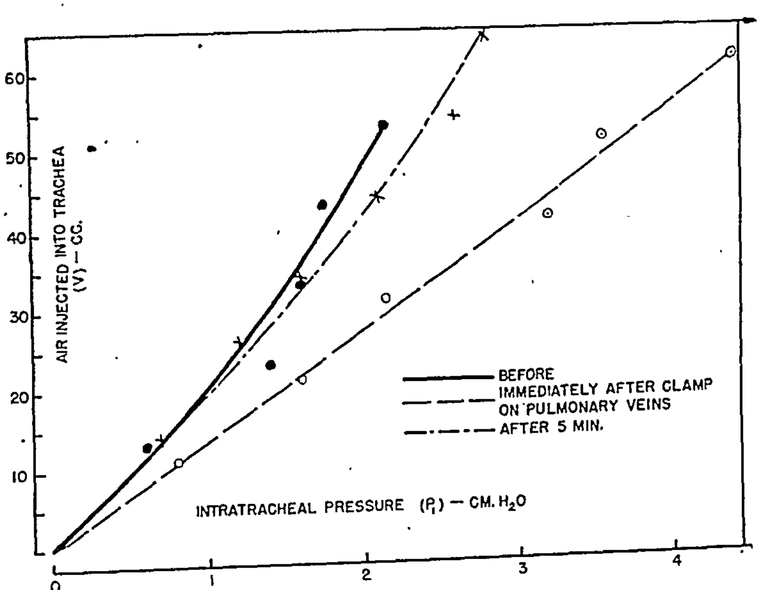

Fig. 5. The effect on pulmonary distensibility of maintaining a clamp on the pulmonary veins for more than five minutes as show in volume/"intrathoracic" pressure curves. A decrease in distensibility of the lung is shown by a decrease in the slope of the curve.

Under special circumstances another interesting observation was made. There was a decrease in distensibility of the lung as reflected by a change in the slope of the volume-pressure curve immediately after the clamp was applied. However, when the clamp was left on the pulmonary veins for as long as 5 minutes, it was noted that the slope of the curve had returned toward the control level (fig. 5). There was thus an apparent increase in distensibility which

occurred after the clamp had been on for 5 minutes or more. At least two possibilities as to the cause for this phenomenon may be considered:

1. The obstruction to the egress of blood from the pulmonary veins when present for a period of 5 minutes or more resulted in a redistribution of blood from small vessels where its presence would affect lung distensibility most, to over-stretched larger vessels where it would have less effect on distensibility.

2. The obstruction to the return of blood from the pulmonary circuit resulted in a marked pulmonary hypertension which imposed a heavy load on the right

TABLE 1

*The effect in a typical experiment of varying pulmonary congestion on the distensibility of the lung**

PROCEDURE IN SEQUENCE	$\Delta V/\Delta P$
1. Control (no clamp)	11.5
2. Clamp on pulmonary veins	9.4
3. Control (no clamp)	12.2
4. Clamp on pulmonary veins	10.7
5. Same clamp left on pulmonary veins for five minutes	13.0
6. Control (no clamp)	21.4
7. Clamp on pulmonary veins	14.0
8. Same clamp left on pulmonary veins for five minutes	21.4
9. Control (no clamp)	17.6
10. Repeat control (no clamp)	17.6
11. Clamp on pulmonary artery	18.2
12. Control (no clamp) after ninety minutes	14.0
13. Clamp on pulmonary veins	10.7
14. Control (no clamp)	15.3

* Distensibility of the lung is proportional to the ratio $\Delta V/\Delta P$. (ΔV is always 60 cc. of air injected intratracheally and ΔP is the intratracheal pressure resulting from this injection of air.)

heart. This could have caused acute failure of the right ventricle leading to a reduction in the pulmonary congestion. Evidence of this marked right ventricular strain was seen in the marked dilatation of the right ventricle which occurred when the clamp was left on for 5 minutes or more. This second factor is probably the more important one.[3]

A clamp placed on the main pulmonary artery trunk with subsequent reduction in the blood in the lungs often resulted in an increased distensibility of the lung. In a similar way almost complete occlusion of the superior and inferior vena cava

[3] It is interesting to note that in a patient with chronic left congestive failure, there is a diminished distensibility of the lung (3) which is present for a long period of time. In such cases the right ventricle usually maintains a normal minute output, in contradistinction to the acute experiments described here where the addition of a sudden tremendous load on the right ventricle produced marked forward failure (reduction in output). A distinction should be made between the transition period, during which a discrepency between the output of the right and left ventricles initiates the pulmonary congestion, and the longer period which follows in which the outputs of both ventricles are equal, the pulmonary congestion being maintained.

resulted in increase of the distensibility of the lung, obviously as a result of the subsequent diminution of blood in the lung. However, on several occasions clamping of the pulmonary artery did not have this effect but produced the opposite changes in the volume-pressure curve. This occurred when temendous dilatation of the right ventricle and right atrium appeared. These dilated chambers resting on the expanding lung could cause, by their added weight, paradoxical increases in intrapulmonary pressure and thus distort the curve.

Occlusion of the ascending aorta conversely resulted in a decrease of distensibility of the lungs by damming blood in the pulmonary circuit.

As the experiment proceeded in each animal, the distensibility of the lung appeared to increase. This may have been due to the shift of blood from the pulmonary reservoir to other regions to counterbalance the shock produced by the anesthesia and surgery connected with the experiment.

The results of these many maneuvers on the distensibility of the lungs in a typical animal are shown in table 1.

SUMMARY

1. The effect of pulmonary congestion on the distensibility of the lungs was investigated in both the isolated lung of the dog and in the open-chest animal. The degree of distensibility was derived from the corresponding increases in intratracheal pressure produced by inflation of the lung with known volumes of air and, in the isolated lung, also from changes produced by lung inflation upon the ratio of intratracheal to "intrathoracic" pressures (the latter being the pressure in the chamber in which the lung was enclosed).

2. In the isolated lung, injection of known volumes of heparinized blood into the pulmonary artery resulted in a diminished distensibility of the lung. Withdrawal of this blood caused a return of the distensibility toward its control level, thus demonstrating that the decreased distensibility was due to intravascular blood rather than intra-alveolar transudate.

3. In the open-chest dog, compression of the pulmonary veins, producing pulmonary congestion, also resulted in a decrease in distensibility of the lung. Release of the compression caused a return of the distensibility toward its control level. Aortic compression, another method for producing pulmonary congestion, also diminished pulmonary distensibility.

4. Improvement in pulmonary distensibility occurred after compression of the main pulmonary artery, after partial compression of both venae cavae, and during the development of shock toward the end of the experiment.

5. This study demonstrated that the distensibility of the lungs varied in an inverse manner with the amount of blood in the pulmonary vessels.

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A QUANTITATIVE STUDY OF ANTIFIBRINOLYSIN IN CHICK PLASMA: INCREASE IN ANTIFIBRINOLYSIN ACTIVITY DURING PTEROYLGLUTAMIC ACID DEFICIENCY[1]

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Various recent studies concerned with fibrinolysin have advanced our knowledge of this important enzyme considerably (1–20). Work on its purification has made available material in concentrated form (3) useful for studying fundamental relationships and for studying the action of antifibrinolysin[2] activity of plasma. It is with this aspect of the general problem that the work herein reported is concerned.

Fibrinolysin is a proteolytic enzyme which is potentially present in plasma. Its precursor, profibrinolysin, has no proteolytic power but may become activated through the action of streptokinase, chloroform and possibly some mechanism in the intact organism which at present is unknown (1). Fibrinolysin is a protease which decomposes fibrinogen and fibrin and also destroys prothrombin (16). It can be derived from bovine plasma in potent concentration (3). Christensen and Macleod (6) have pointed out that it has no relationship to trypsin.

Antifibrinolysin inactivates fibrinolysin and is present in the plasma of all species so far tested (12). We have found that plasma may be sufficiently diluted so that at equilibrium the low concentration of antifibrinolysin has inactivated only a part of the fibrinolysin in the substrate. The quantitative assay of antifibrinolysin depends upon the measurement of the amount of active fibrinolysin remaining after equilibrium is reached. On the basis of this approach we have found that antifibrinolysin activity increases in the plasma of chicks fed a pteroylglutamic acid deficient diet. The crystalline vitamin prevents this increase.

METHODS. *Imidazole buffer.* One and seventy-two hundredths grams of imidazole were dissolved in 90 cc. of 0.1 N. HCl. The pH was adjusted to 7.25 with concentrated HCl or NaOH and the mixture was then diluted to 100 cc. volume with water.

Fibrinogen. To insure precision and accuracy in assays the preparation and standardization of the reagents are extremely important. Fibrinogen must be of high purity and relatively stable. The fibrinogen used in these studies was prepared by the method which we have described (21). A 0.5 per cent to 1 per cent solution is prepared in a 2 per cent saline solution containing 5 per cent by

[1] Aided by a grant from the U. S. Public Health Service.

[2] There has been discussion concerning nomenclature (1, 3, 6, 30). In this paper we follow the suggestions of Loomis *et al.* (3) and Astrup and Permin (1). In previous reports from this laboratory (16, 17) the term fibrinolysin inhibitor was used in place of the term antifibrinolysin.

volume of imidazole at pH 7.2. The salt in excess of physiological concentration increases the stability of the fibrinogen. The concentration of the latter is determined by an analysis of the tyrosine content (21, 22). The solution is then poured into test tubes, each tube receiving a sufficient quantity to complete the average number of assays required in one day. The fibrinogen solution is quick frozen in a dry ice alcohol mixture and stored at −20°C. If these precautions are followed, the fibrinogen will remain stable. In preparation for assays the fibrinogen is thawed without agitation at 40°C. in a water bath. When fluid, the stock solution is diluted to a 0.2 per cent fibrinogen concentration and the saline concentration adjusted to approximately 0.9 per cent. The tube containing the 0.2 per cent fibrinogen is maintained at 40°C. in a water bath throughout the period of use. The clottability remains unchanged for periods up to 24 hours if the indicated conditions relating to temperature and salt concentration are maintained.

Thrombin. Thrombin topical, Parke, Davis & Company, was used. It was dissolved in 50 per cent glycerol-saline to give a solution containing 2,000 Iowa units per cc.(23).

Standardized fibrinolysin solution. The material was obtained from bovine plasma by the methods described in detail (3). In preparation for use it is dissolved directly in imidazole buffer. More than the amount required for assays covering a 3 hour period should not be dissolved at one time since thereafter the activity gradually decreases. The fibrinolysin is allowed to stand from 20 minutes to 1 hour before standardization since during the first 15 to 20 minutes after the solvent is added the fibrinolytic activity increases. Its concentration must be standardized in terms of activity. To 0.2 cc. of fibrinolysin solution is added 0.2 cc. of 0.2 per cent fibrinogen solution. A stopwatch is started. The solutions are quickly mixed and thrombin is added by stirring rod. The stirring rod, 3 to 4 mm. in diameter, is immersed in the thrombin solution, removed and inserted with a rotating motion in the fibrinolysin-fibrinogen mixture and withdrawn. With practice the thrombin can be added within 2 to 3 seconds. The reaction tube is placed in a water bath at 28°C. until lysis is complete. A firm clot forms within 15 seconds. The end-point is the time at which the contents of the tube become completely fluid and the solution runs freely, with no gel-like portions, down the sides of the tube as it is tilted. At the end point the stopwatch is stopped and the time interval recorded in seconds. The tube must be handled gently in determining the end-point, because shaking tends to disturb the reaction and may result in a false end-point. All determinations are carried out in triplicate and are based on the average for the 3 measurements. The fibrinolysin concentration is adjusted to give a lysis time of 120 ± 5 seconds.

Double strength standardized fibrinolysin solution. This solution is made exactly as the standardized fibrinolysin solution except that the concentration of fibrinolysin is doubled.

Chicken plasma. Blood was obtained from the carotids, mixed with 1.85 per cent potassium oxalate in the proportion 7 to 1. It was immediately centrifuged.

The hematocrit was noted. The plasma was defibrinated by adding solid thrombin and whipping out the fibrin. If tests could not be completed the same day the defibrinated plasma was stored at −20°C. All data are calculated to oxalate free plasma.

Fibrinolysin unit. Loomis, George and Ryder (3) define a unit of fibrinolysin as that amount which will dissolve 1 ml. of a 0.3 per cent fibrin clot in 120 seconds at pH 7.2 and 45°C. in an isotonic saline system buffered with imidazole. Because we have found that fibrinolysin rapidly loses activity in solution at 45°C. we believe it is essential to reduce the temperature to 28°C. The fibrinogen concentration has been reduced to 0.1 per cent. This concentration produces a firm clot which rapidly collapses when lysis is nearly complete. Thus a sharp end-point is insured. The net result is that a unit as employed here in-

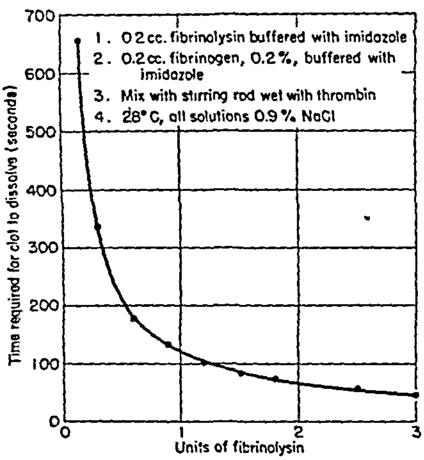


Fig. 1

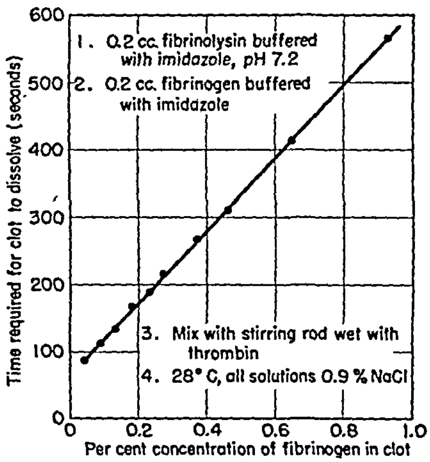


Fig. 2

Fig. 1. Relationship between fibrinolysin concentration and clot dissolving time. Only fibrinolysin concentration was varied.

Fig. 2. With fibrinolysin concentration remaining constant the time required for the clot to dissolve is directly proportional to fibrin concentration.

volves more fibrinolysin. It requires approximately 5 times more than the amount in the unit described by Loomis *et al.* (3).

EXPERIMENTAL. *Lysis of fibrin clot.* Figure 1 shows the relationship between the time required for a clot to dissolve and fibrinolysin concentration. It requires very potent fibrinolysin concentrations to dissolve the clot in a few seconds, and by comparison only minute amounts are required to accomplish the same result in time intervals involving minutes. These latter clot resolution times are the ones described commonly in the literature, because potent preparations of fibrinolysin were not available until recently. Many conclusions were based on the study of minute traces of this enzyme.

If the data in figure 1 are plotted on log projection paper approximately a straight line is obtained. It is interesting that the curve recalls the relationship between fibrinogen clotting rate and thrombin concentration (28). The logarithm of clotting time is inversely proportional to the logarithm of thrombin concentra-

tion, and similarly the logarithm of clot dissolving time is inversely proportional to the logarithm of the fibrinolysin concentration.

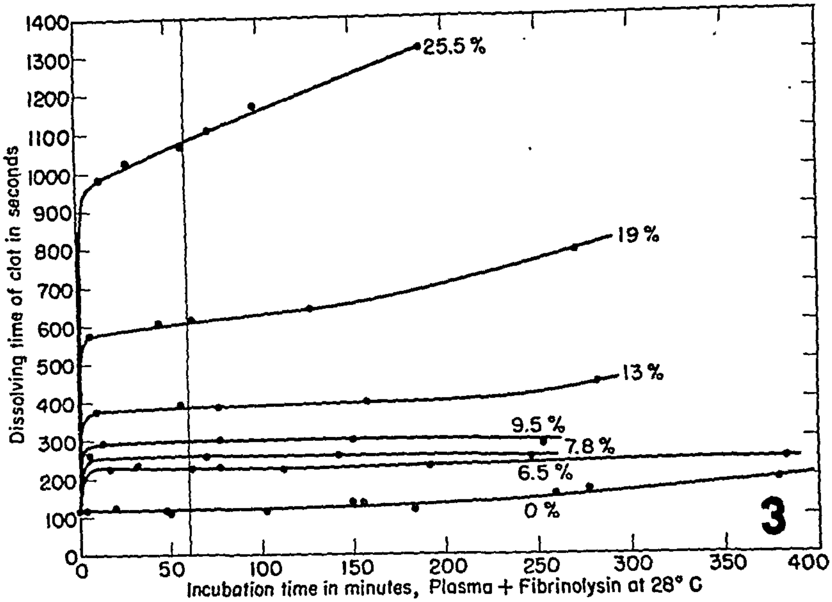
Fibrinogen concentration and lysis time. In these experiments the fibrinogen concentration was the only known variable. With a fixed concentration of fibrinolysin the lysis time became progressively longer as the fibrinogen concentration was increased. Figure 2 shows that the relationship between lysis time and fibrinogen concentration is a straight line function. It is thus important to control fibrinogen concentration in fibrinolysin assay procedures. Additional data, not included in the figure, indicate that the straight line function extends through a 2 per cent fibrinogen concentration.

Inactivation of fibrinolysin with antifibrinolysin. If a standardized fibrinolysin solution is prepared as described above the stability characteristics are favorable. Only slight decreases in activity are noted in a period of hours. If, however, a small amount of chick plasma is first mixed with the fibrinolysin the latter is destroyed by the antifibrinolysin of the plasma. The significant finding is that only a portion of the fibrinolysin is destroyed and the reaction reaches *equilibrium.* This type of reaction could hardly have been predicted from available information in the literature. It might be pointed out, however, that plasma does not inactivate unlimited quantities of thrombin (23), thromboplastin reaches equilibrium when it reacts with an excess of prothrombin (24), and there is evidence that fibrinolysin will not destroy unlimited quantities of prothrombin (17). In fact there is now ample evidence that the interaction of proteins involved in the blood clotting mechanism commonly proceeds to equilibrium. Although it is a simple concept little imagination is required to realize how these circumstances may have caused confusion in the past.

As shown in figure 3, the maximum amount of fibrinolysin inactivated by the antifibrinolysin in diluted chicken plasma occurs within 5 minutes. Thereafter the residual fibrinolysin activity remains constant. The difference between the original quantity of fibrinolysin and that which remains represents a quantitative measure of antifibrinolysin activity. As a matter of practical convenience the calculations for antifibrinolysin activity are best made on the basis of equilibrium conditions found after antifibrinolysin has been incubated with fibrinolysin for 60 minutes.

Units of antifibrinolysin in chicken plasma. Figure 4 graphically depicts the relationship between chicken plasma dilutions and the dissolving time of standard fibrin clots containing an initial fibrinolysin concentration of 1 unit. The plasma used was the pooled plasma of 6 one year old hens. Five units of chick plasma antifibrinolysin are defined as the quantity of antifibrinolysin which within 60 minutes at 28°C. and at pH 7.2 will inactivate 50 per cent of the fibrinolysin in 1 cc. of a standard solution containing 1 unit of fibrinolysin per cc. To establish units for chicken plasma antifibrinolysin, the dissolving time of $\frac{1}{2}$ unit of fibrinolysin was measured on the curve in figure 1. This was 210 seconds. The dissolving time of 210 seconds was then projected from the curve in figure 4 to the ordinate. Five chick antifibrinolysin units were therefore assigned to the point on the figure 4 curve which corresponds to the 210 seconds dissolving time.

The ordinate was then marked off in multiples and fractions of 5 antifibrinolysin units. To calculate antifibrinolysin units, the co-ordinate is extended from the abscissa to intersect the curve in figure 4 and the horizontal co-ordinate is ex-



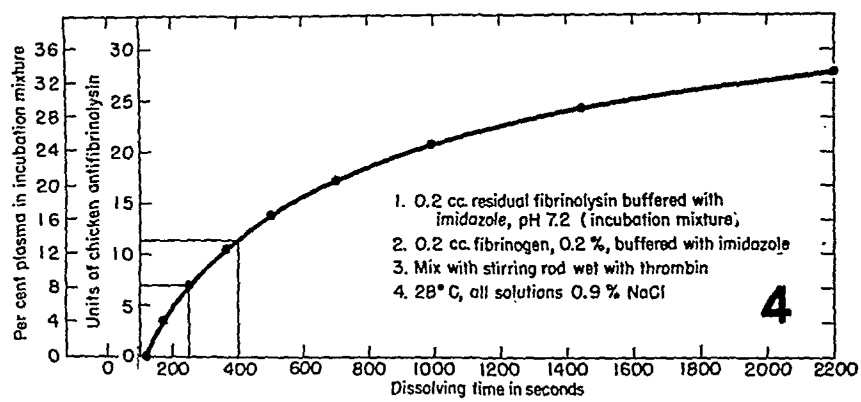


Fig. 3. Double strength standardized fibrinolysin solution was mixed with an equal volume of variously diluted plasma for periods of time (X-axis). The remaining fibrinolysin activity was then ascertained by measuring the time required (Y-axis) to dissolve standard clots. The reaction between fibrinolysin and antifibrinolysin is rapid and equilibrium is established. The amount of fibrinolysin destroyed is a quantitative measure of antifibrinolysin activity. The 60 minute co-ordinate is especially indicated, because this time interval is used in the assay of antifibrinolysin.

Fig. 4. The quantity of residual fibrinolysin (that which remains after antifibrinolysin has destroyed fibrinolysin and equilibrium is established) is indicated as dissolving time in seconds. From the dissolving time the units of antifibrinolysin can be noted on the Y axis.

tended to meet the unit scale on the ordinate. The unit value per cubic centimeter is obtained by multiplying the number of units read off from the curve by the dilution factor. Correction is also made for oxalate.

Example: Antifibrinolysin assay. Place 0.1 cc. of double strength standardized fibrinolysin solution in a test tube (50 × 8 mm. I.D.). Add 0.1 cc. diluted plasma (antifibrinolysin). Incubate at 28°C. for exactly 1 hour. Add 0.2 cc. of fibrinogen (0.2 per cent) solution. Start stopwatch and immediately add thrombin with stirring rod as described above. Note lysis time in seconds. If lysis occurs in 120 seconds there is no antifibrinolysin present. If the lysis time is longer, say 210 seconds, then the antifibrinolysin concentration of the diluted plasma is 5 units per cc. (cf. fig. 4). It is recommended that dilutions of plasma be made so that lysis will occur in 400 seconds or less.

Antifibrinolysin and anemia. Preliminary studies of the plasma of human pernicious anemia patients revealed that the antifibrinolysin activity is greatly increased above the normal value. It was, therefore, indicated that macrocytic anemia (25) be produced in chicks and that the effect of crystalline pteroylglutamic acid be studied. Since Campbell, Brown and their associates (26, 27) were actively engaged in the study of crystalline vitamin B_c we requested that they furnish us plasma from their chicks for our study. The handling of their birds and composition of the diets is described in detail (26, 27). Five diets were used as follows:

No. 1. Fortified broiler ration. Larro Milling Co. + 3 per cent desiccated pork liver + 3 per cent dried brewer's yeast.

No. 2. Synthetic diet, no. 59744, table 1, Campbell *et al.* (27), supplemented with 500 gamma of pteroylglutamic acid per 100 grams' ration.

No. 3. Synthetic diet, same as no. 2 except that casein was extracted with hot alcohol.

No. 4. Synthetic diet, same as no. 2 except that pteroylglutamic acid was omitted.

No. 5. Synthetic diet, same as no. 4 except that casein was extracted with hot alcohol.

The chicks were fed the respective diets for 28 days after hatching. Blood drawn on the 28th day was centrifuged to remove the cells and the plasma was defibrinated by adding commercial thrombin and whipping out the fibrin. Weight gains and hematocrit values were similar to those previously described (27, 28). Blood studies were not made but may be assumed to parallel those reported for chicks under similar experimental conditions (28).

The data obtained are presented in table 1. In the first experiment a pooled sample of plasma from 2 chicks on the vitamin deficient diet was found to contain almost twice as much antifibrinolysin as the chicks receiving the same diet together with the vitamin, or chicks receiving a broiler ration. The latter ration is believed to be adequate in all respects. A second experiment was then performed. In this group 4 chicks on a synthetic vitamin B_c free diet had about twice the inhibitor concentration found in chicks receiving the same diet fortified with crystalline pteroylglutamic acid. For our third study the first experiment was repeated, but with a larger number of chicks; and the data confirm the first work.

In all cases there is a tendency for antifibrinolysin concentration to be lowest in the plasma from chicks on the fortified broiler ration. This may mean that more vitamin would be required on the synthetic diet to establish normal values for antifibrinolysin. However, the important point which we consider to be

amply established from the data in table 1 is that the antifibrinolysin concentration increases in plasma as the result of pteroylglutamic acid deficiency.

TABLE 1

Antifibrinolysin concentration in chicken plasma

DIET*	NUMBER OF CHICKS	ANTIFIBRINOLYSIN, UNITS PER CC.	
		Plasma	Whole blood
Experiment no. 1			
No. 2 synthetic, fortified with pteroylglutamic acid	1	119	81
	1	124	87
	1	168	116
	1	108	75
No. 4 synthetic, pteroylglutamic acid free	2 pooled	210	191
No. 1 fortified broiler ration	1	86	61
	1	117	81
	1	87	61
Experiment no. 2			
No. 3 synthetic, fortified with pteroylglutamic acid	1	128	86
	1	158	104
	1	148	100
	1	142	97
No. 5 synthetic, pteroylglutamic acid free	1	266	157
	1	250	188
	1	323	259
	1	431	294
Experiment no. 3			
No. 2 synthetic, fortified with pteroylglutamic acid	6	168	109
	5	158	104
	7	149	97
No. 4 synthetic, pteroylglutamic acid free	9	216	176
	5	203	187
No. 1 fortified broiler ration	3	97	65
	2	99	66
	2	104	67

* Consult text for detail and also (26, 27).

DISCUSSION. The mechanism responsible for the production of an increased antifibrinolytic activity in the blood of the pteroylglutamic acid deficient chick must temporarily be conjectural. Possibly hypoxia and tissue injury permit the release of excessive profibrinolysin or an activator of profibrinolysin which in turn may stimulate the organism to the production of more antifibrinolysin. On the other hand it is conceivable that protoplasmic disintegration or injury to cell membranes may permit the direct release of antifibrinolysin with or without a rise in fibrinolysin or fibrinolysin activator. It may also be hypothesized that altered function in anemia of such organs as the bone marrow, liver or spleen play a rôle in the formation of an increased antifibrinolytic activity, although the

probability is remote since in other pathologies which do not specifically effect these organs antifibrinolysin activity is also increased (29).

Quantitative assay of antifibrinolysin may have practical value. Preliminary studies of the plasma of human pernicious anemia patients reveal that the antifibrinolytic activity is greatly increased above the normal value as in chicks. Additional observations indicate that the assay of antifibrinolytic activity in human patients may be a sensitive index of effective therapy, since in two pernicious anemia patients who were followed during therapy the antifibrinolysin activity significantly decreased before a definite change could be found in the cellular picture.

SUMMARY

When fibrinolysin dissolves fibrin clots the logarithm of the time required is inversely proportional to the logarithm of the fibrinolysin concentration. The amount of fibrin substrate also affects lysis time. Greater fibrin concentrations require longer lysis times and the relationship is a straight line function. When fibrinolysin is inactivated with a small amount of antifibrinolysin (diluted plasma) the inactivation reaction comes to equilibrium in less than 10 minutes. Active fibrinolysin remains. The difference between the original fibrinolysin concentration and the concentration at equilibrium is a quantitative measure of antifibrinolysin activity. This basic relationship has been adapted to the quantitative measurement of antifibrinolysin activity in plasma.

Macrocytic anemia in chicks, resulting from pteroylglutamic acid deficiency, is accompanied by an increase in plasma antifibrinolysin activity. Crystalline pteroylglutamic acid prevents this marked increase.

We wish to thank C. J. Campbell and R. A. Brown for supplying us with plasma from chicks receiving their experimental diets, and Eugene C. Loomis for generous supplies of fibrinolysin. We also wish to thank Parke, Davis & Company for supplying funds for Research in Physiology.

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ON THE MECHANISM OF THE "SPONTANEOUS" RE-INNERVATION IN PARETIC MUSCLES

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Almost simultaneously it has been reported from three different laboratories (Wehrmacher and Hines, 1945; van Harreveld, 1945; Hines, Wehrmacher and Thomson, 1945; Weiss and Edds, 1946) that changes may take place in paretic muscles, which result in a considerable and sometimes even complete restoration of the muscle force a few months after a partial denervation, performed by a technique which excludes regeneration of the nerve supply. The possibility that hypertrophy of the muscle fibers which escaped denervation could alone account for this increase of muscle force was rejected, and the re-innervation of the muscle fibers which had lost their normal nerve supply was considered as the chief cause for the improvement of muscle function. The most convincing evidence for the efficacy of this mechanism was provided by the scarcity of degenerated muscle fibers in histological preparations of muscles from which a considerable portion of the nerve supply had been removed some months before (3, 5). Unattached terminal branches ending freely in the muscle have been considered as the source of the reinnervation of the orphaned muscle fibers (1, 5). However, in view of the magnitude of the functional improvements observed, this factor can be of but minor importance, and the active increase of the terminal branching of the motor nerve fibers which escaped degeneration is believed to be the principal mechanism involved.

In view of the above considerations it was of interest to look for mechanisms which might stimulate the motor nerve fibers to form a more luxuriant branching. It seemed not unlikely that this stimulus would be related to the degenerative changes occurring in the muscle fibers which lost their nerve supply in the partial denervation. The increase of the terminal branching might for instance be caused by products formed in the degenerating muscle fibers. The present investigation is an attempt to find experimental evidence for such a chemical stimulus.

METHODS. The sartorius muscle of the rabbit was used throughout this investigation. The m. sartorius is a thin, narrow muscle situated superficially on the anterior and medial side of the thigh. The muscle is innervated by the n. saphenous, a branch of the n. femoralis (fig. 1, I). The 5th and 6th lumbar segments are usually the only segments participating in the innervation of the sartorius, L6 in most rabbits supplying the larger part of the nerve supply (3). Pulling the 6th lumbar spinal nerve out of the spinal cord thus causes in most rabbits a severe paresis of the m. sartorius. In all experiments of this investigation the two sartorius muscles of the animal were prepared for isometric record-

ing, two weeks after the partial denervation of one of them by pulling L6 out of the spinal cord. On both sides the nerve branch carrying the contribution of L5 for the m. sartorius was dissected and stimulated faradically. The force developed on the normal side served in this way as a control for the strength of contraction on the operated side. The relation between these forces was determined independently of the absolute tension developed, which varies considerably

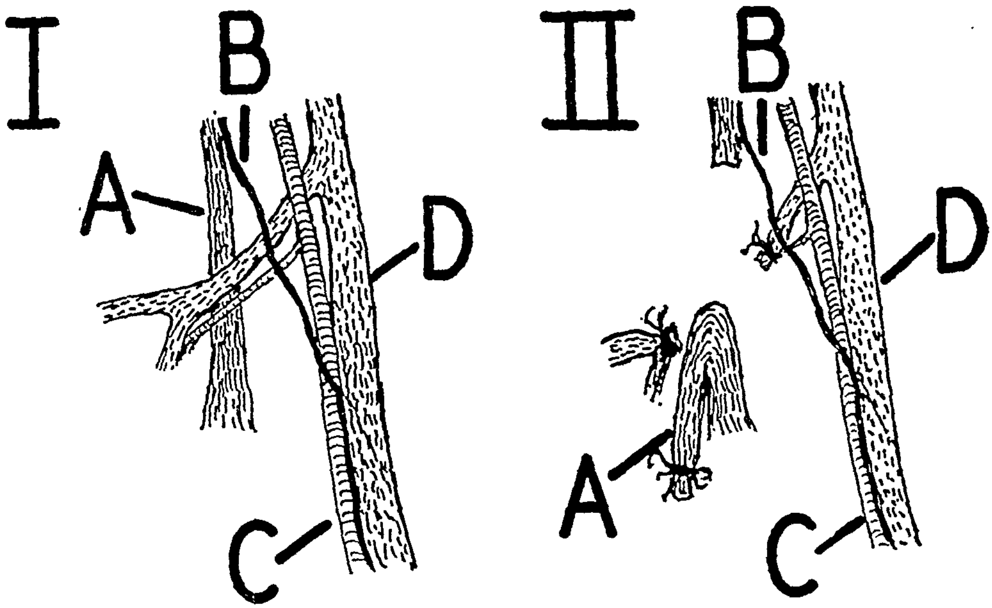

Fig. 1. *A*, femoral nerve. *B*, n. saphenous. *C*, femoral artery. *D*, femoral vein.

I shows the anatomical relation between the femoral and saphenous nerves and the blood vessels. The saphenous nerve crosses over the arterial and venous branches running laterally, whereas the part of the femoral nerve for the m. quadriceps runs under these branches.

II shows the operation used for the denervation of the m. quadriceps. The branches of the femoral artery and vein are doubly ligated and severed. The femoral nerve is ligated and severed near the junction with the n. saphenous. The femoral nerve is then dissected distally and fastened to the fascia.

from animal to animal, by computing the quotient, Q, of the difference in force between the operated and the control side, divided by the smaller of the two.

$$Q = \frac{F(\text{operated}) - F(\text{control})}{F(\text{smaller of two})}$$

A positive Q thus indicates a functional improvement of the partially denervated muscle. It was shown before (3) that the Q after 2 to 6 months of re-innervation depends greatly on the degree of initial denervation. Although this influence is probably of less importance when the re-innervation is allowed to proceed for only two weeks, it seems indicated to compare only groups of experiments in which the mean forces on the control sides do not differ too much.

The above methods have been described in detail in a previous communication (3).

RESULTS. *Re-innervation during the first two weeks after partial denervation.* In 24 rabbits the 6th lumbar spinal nerve was removed unilaterally. Two weeks later the contractions of the operated and normal sartorius muscles during stimulation of L5, were compared. The results are given in table 1A. The mean force developed on the normal side was 57 grams, on the operated side 80 grams. The mean Q was +0.6, with a standard error of 0.3. It has been shown previously (3) that, although considerable individual differences are sometimes found in normal rabbits between the right and left sartorius contractions elicited by stimulation of L5, there is statistically no difference. The mean Q of + 0.6 (statistically significant on the 5 per cent level), thus indicates that the "spontaneous" re-innervation has started 2 weeks after partial denervation. This result is in accordance with a previous finding based on a smaller number of animals (3).

TABLE 1

Comparison of the force developed in the right and left sartorius muscles by stimulation of L5 under various experimental conditions

EXPERIMENTAL CONDITION	NO. OF EXPTS.	MEAN MUSCLE FORCE IN GRAMS		QUOTIENT Q	
		Normal side	Operated side	Range	Mean
A. 2 weeks after removal of L6 on one side	24	57	80	−3.0 to +3.5	+0.6 ± 0.3*
B. 5 to 6 weeks after denervation of m. quadriceps; 2 weeks after removal of L6 on that side	25	51	90	−0.3 to +4.8	+1.3 ± 0.3
C. 8 weeks after denervation of m. quadriceps	22	58	58	−2.0 to +2.5	+0.1 ± 0.2
D. 6 weeks after cutting sciatic nerve; 2 weeks after removal of L6 on that side	22	38	54	−1.4 to +3.1	+0.7 ± 0.2
E. 3 weeks' application of extract of degenerated muscle; 2 weeks after removal of L6 on that side	31	33	57	−2.2 to +6.0	+1.5 ± 0.3
F. 3 weeks' application of extract of normal muscle; 2 weeks after removal of L6 on that side	32	36	49	−3.1 to +2.9	+0.5 ± 0.2

* Standard error.

Effect of contact with degenerating muscle on the spontaneous re-innervation. The largest portion of the sartorius muscle of the rabbit is in close contact with

the m. vastus medialis, part of the quadriceps muscle. The conditions for the transport of products from one muscle to the other therefore are excellent. Use has been made of this anatomical relation to test formation in degenerating muscle of products influencing the spontaneous re-innervation of paretic muscle.

The quadriceps muscle can be denervated conveniently by cutting the femoral nerve, leaving the n. saphenous intact. To prevent regeneration, the distal stump of the femoral nerve was dissected over some distance and was sewn to the fascia as far away from the proximal stump as possible (fig. 1, II). Three to 4 weeks were allowed for the formation of the hypothetical products in the degenerating m. quadriceps and for their diffusion into the m. sartorius. Then in a second operation the 6th lumbar spinal nerve was pulled out of the cord on the same side of the animal. An interval of two weeks between the partial denervation and the final appraisal of the muscle force of the normal and operated m. sartorius was chosen, because, although the spontaneous re-innervation has started at this time it has not proceeded very far, and the effect of any procedure enhancing this process should be most apparent at this stage.

This experiment was performed in 25 rabbits. The mean force developed on the normal side was 51 grams, on the operated side 90 grams (table 1B). The mean Q was $+ 1.3$, with a standard error of ± 0.3. These values can be compared with the mean Q value of $+ 0.6 \pm 0.3$ (table 1A) found in the control group. The mean Q in the experiment in which the sartorius was subjected to the influence of the degenerating m. quadriceps was thus about twice as large as the Q of the control group. The statistical evaluation of these two experiments, however, shows that the difference found, though suggestive, does not quite reach statistical significance on the 5 per cent level ($t = 1.75$).

Effect of denervation of the quadriceps muscle on the contraction of the m. sartorius. The enhanced improvement in function of the paretic m. sartorius, induced by denervation of the quadriceps muscle (table 1B), might be due to changes other than enhanced re-innervation, for instance to hypertrophy of the functioning muscle fibers. This possibility was examined in the following experiment. In 22 rabbits the femoral nerve was severed in the way described above, leaving the n. saphenous intact. Two months later the contractions in the sartorius muscles elicited by the faradic stimulation of L5 on the normal and operated side were compared. The average force on both sides was 58 grams and the average Q was $+0.1$ with a standard error of 0.2 (table 1C). It is obvious from this experiment that the denervation of the quadriceps muscle alone does not change the force of the sartorius muscle during stimulation of L5 and there thus is reason to believe that the enhanced improvement found in the group of experiments of table 1B is an effect of the degenerating m. quadriceps on the re-innervation process in the m. sartorius.

Effect of severing the homolateral sciatic nerve on the spontaneous re-innervation. This series of experiments was concerned with the effect of the denervation of a muscle group at least as large as the m. quadriceps but not in close proximity to the sartorius muscle on the spontaneous re-innervation of the latter. In 22 rabbits the sciatic nerve was severed at the place where it leaves the pelvis and a

few centimeters of this nerve were resected. This operation causes the degeneration of the muscles of the shank and of the flexor muscles of the thigh from which the m. sartorius is separated by the adductor muscles and the m. gracilis, innervated by the obturator nerve. Four weeks later the partial denervation of the sartorius muscle was performed by pulling the 6th lumbar spinal nerve out of the cord, and after two more weeks the force of the normal and partially denervated m. sartorius was compared during the faradic stimulation of L5. The mean force developed on the normal side was 38 grams, on the operated side 54 grams (table 1D). The mean Q was $+0.7$ with a standard error of 0.2. Since this Q is practically the same as found in the control group of table 1A, it can be concluded that the spontaneous re-innervation in the m. sartorius is not influenced by degeneration in the muscles innervated by the sciatic nerve.

Effect of an extract of degenerated muscle on the spontaneous re-innervation. The four series of experiments described suggest that the spontaneous re-innervation of a paretic muscle is caused or is at least enhanced by chemical agents produced by degenerating muscle. The fact that the enhancement could be demonstrated only when the degenerating muscle was in close proximity to the m. sartorius would indicate that the products are not transported by the bloodstream, and can diffuse only over short distances. They might be fat soluble substances comparable as far as their mode of transportation is concerned with the neuro-humors responsible for color changes in fish (Parker, 1936).

To investigate this possibility, the effect of an ether extract of degenerating muscle on the spontaneous re-innervation was examined. Degenerating muscle was obtained from rabbits in which the spinal cord was removed caudal of L3 to L4. In such animals the entire musculature of the hind legs and around the pelvis is denervated. Two weeks after the operation 150 to 200 grams of degenerating muscle could be collected per large rabbit. This amount of muscle was frozen in dry ice and was crushed, then extracted with 300 cc. of peroxide-free ether under constant shaking for 24 hours. In the course of this period the ether was renewed once. The ether was then distilled off, yielding (per rabbit) 2 to 3 grams of an oily substance containing a white precipitate. An amount of the extract corresponding to 75 to 100 grams of degenerated muscle was injected into the fascia covering the sartorius muscle, thereby bringing the extract in the closest possible contact with the muscle. One week later this muscle was partially denervated by pulling the 6th lumbar spinal nerve out of the cord, and two weeks after this the muscle force of the two sartorius muscles of the animal was determined by stimulating L5.

Three weeks after the application of the extract to the sartorius muscle it had in general not been completely resorbed. It had in most cases caused a considerable inflammatory reaction, evidenced by a marked hyperemia.

This experiment was carried out on 31 rabbits; the results are given in table 1E. The mean force on the normal side was 33 grams, on the operated side 57 grams. The mean Q was $+1.5$ with a standard error of 0.3. The mean Q in this experiment thus was more than twice as large as that of the control group of

table 1A, and as that of the group of experiments compiled in table 1D, which can also be considered as a control group. In this latter group the mean force of the control muscles is more equal to the corresponding value in the experiment in which the extract was used (table 1E). These observations suggest that the extract has an enhancing effect on the spontaneous re-innervation; the differences, however, are statistically not quite significant ($t = 1.9$ for the experiments of table 1A and 1E, and $t = 1.8$ for those of table 1D and 1E).

Effect of an extract of normal muscle on the spontaneous re-innervation. In another series of experiments an ether extract of normal muscle, made in exactly the same way as described above, was deposited on top of the sartorius muscle of 32 rabbits. Again after one week this sartorius muscle was partially denervated and 2 weeks later its muscle force during stimulation of L5 was compared with that of the m. sartorius of the other side.

The extract of the normal muscle caused the same irritation and hyperemia as that of degenerated muscle. The results are shown in table 1F. The mean force developed on the normal side was 36 grams, on the treated side 49 grams. The mean Q was $+0.5$, the standard error 0.2. The mean Q in this experiment is thus slightly smaller than that of the control groups of table 1A and 1D. It is possible that this is an expression of the inflammatory damage to the muscle caused by the extract. An examination of the statistical significance of the difference between the Q of the series treated with the extract of degenerated muscle, and that of this control series in which extract of normal muscle was used, showed that the difference is at least significant on the 5 per cent level ($t = 2.5$).[1] The enhancing effect of the extract of degenerating muscle on the re-innervation thus seems to be lacking in extract of normal muscle.

CONCLUSION AND SUMMARY

The six groups of experiments described above suggest that re-innervation of the paretic sartorius muscle is enhanced by close contact with degenerating muscle or with an extract of degenerating muscle. The enhancement in general is small, which is not surprising as it is obtained against a background of "spontaneous" re-innervation. This, with the considerable variability, makes it difficult and laborius to obtain sufficient data which show statistically significant differences. However, other criteria support these conclusions. The largest Q's are found in the series in which the sartorius muscle had been subjected to the effect of degenerating muscle (table 1B) or to the extract of such muscle (table 1E). Besides the number of negative Q's in these series is considerably smaller than in the control series.

The differences between the effect of the extract of degenerated and of normal muscle indicates that the degenerating muscle forms specific products, which cause, or at least enhance, the "spontaneous" re-innervation. These products

[1] I am indebted to Mrs. H. Bonner who applied the method of covariance to the data of table 1E and 1F, and showed that the mean square for the adjusted means divided by the mean square for error, yields a value for F greater than that at the 1 per cent point.

seem to be absent or less abundant in normal muscle. In this way the degenerating muscle fibers in a paretic muscle would themselves produce some of the factors in the chain of events leading to the recovery of their function.

I am greatly indebted to Mrs J. Wiersma and Miss R. E. Estey for valuable assistance.

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THE BLOOD URIC ACID AND ALLANTOIN OF THE RAT AFTER NEPHRECTOMY AND HEPATECTOMY[1]

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Mann and his associates (1, 2, 11) were able to demonstrate that the liver of the dog was an essential agent in the destruction of blood uric acid. Their work has been confirmed in the rabbit by McMasters and Drury (10) and by Svedberg, Maddock and Drury (13). Maddock and Svedberg (9) found similar changes following total removal of the liver in monkeys. Belief in the hepatic conversion of uric acid to allantoin in subprimate mammals has been based upon three experimental observations: *a*, an increase in blood and urinary uric acid following hepatectomy (2) and a corresponding decrease in urinary excretion of allantoin; *b*, the absence of a rise in blood uric acid after nephrectomy (1), this constituent *presumably* being converted to allantoin by the still intact liver, and *c*, the presence of the enzyme uricase in the liver of such animals (4, 7, 14). Technical difficulties previously prevented the determination of allantoin in the blood of animals subjected to these surgical procedures. It was therefore impossible to quantitate the relationship of the liver to blood allantoin. Relatively little is known about the blood allantoin even of normal animals, since only recently have there appeared adequate methods for the assay of small samples.

In the present report concomitant determinations have been made of the blood uric acid and allantoin in rats before and after nephrectomy, hepatectomy, and nephrectomy followed by hepatectomy. Our results suggest that the liver of the rat functions to convert uric acid to allantoin as the end product of its purine metabolism.

Methods. Male albino laboratory rats of the Wistar strain, six to eight months old and weighing an average of 287 grams were maintained on a diet of Purina Dog and Laboratory Chows with occasional supplement of green vegetables. Determinations of blood uric acid and allantoin were made on 72 normal rats; on 42 rats after bilateral nephrectomy; on five rats after total hepatectomy; and on three rats after nephrectomy followed 15 hours later by total hepatectomy.

Blood samples of approximately 1 ml. volume were obtained by cutting the tail of the ether-anesthetized animal, or by cardiac puncture.

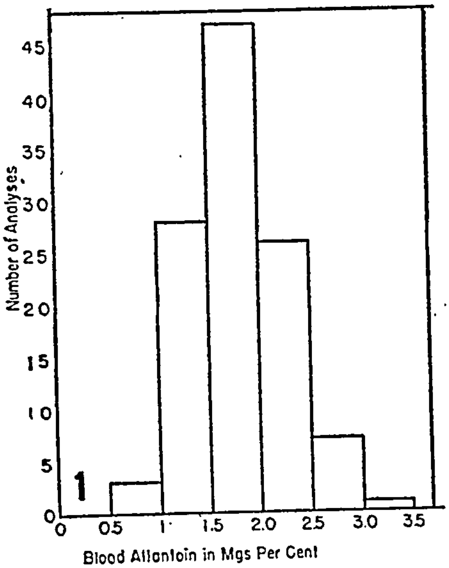
Kidneys were removed through short bilateral lumbar incisions. Blood samples were taken before and at 6, 15, 24, 48 and 72 hours after nephrectomy. As many as four samples were taken from the same animal. Post-operatively the rats received water but no food.

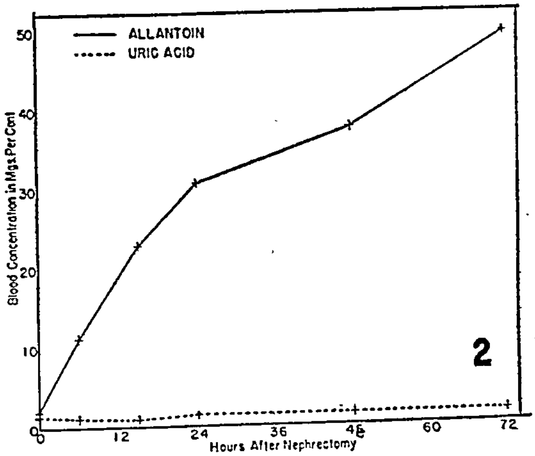
Rats were hepatectomized after total viscerectomy. It was found best to remove the liver by ligating each lobe near its pedicle with soft wool yarn, taking

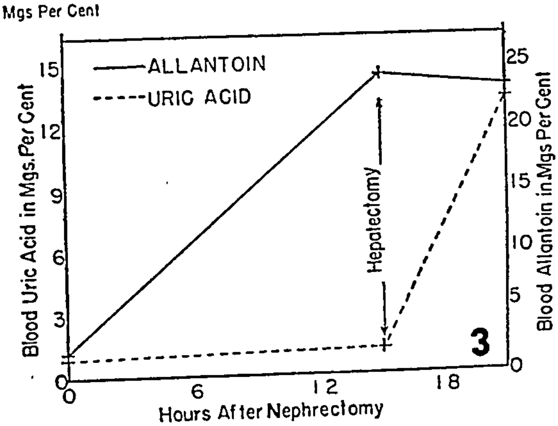
[1] Aided by a grant from the Public Health Service.

care to avoid including the inferior vena cava. These animals were maintained for six hours by hourly subcutaneous injections of 2.5 to 5 ml. of 10 per cent glucose.

Uric acid was determined by the macro method of Folin (6) on an aliquot of the unlaked blood filtrate.







Figs. 1. Histogram of blood allantoin results of 113 analyses on samples from 72 rats. Median 1.8 mgm. per cent; mean 2.1 mgm. per cent; standard deviation ± 0.53 mgm. per cent, computed from the formula $\sigma = \sqrt{\frac{\Sigma\ (x^2)}{N}}$

Fig. 2. The effect of nephrectomy upon the blood allantoin and uric acid of 42 rats.

Fig. 3. The effect of nephrectomy followed by hepatectomy upon the blood allantoin and uric acid of three rats.

Allantoin was determined according to the technique of Christman *et al.* (3) modified in that an unlaked blood filtrate instead of a laked filtrate was used, thus avoiding interference from ergothioneine which remains in the unlaked cells (5). An ice-salt bath at −8 to −10° C. was used in the allantoin assay instead of the 0° bath suggested by Christman *et al.* A yeast blank and two standard

solutions were run concurrently with almost all assays. The standards and blank were manipulated in the same fashion as samples; their values as read on a Klett-Summerson photoelectric colorimeter (12) varied only slightly from day to day. In our hands the correction for uric acid was about $\frac{1}{5}$ of the value of an equal concentration of allantoin, in the range from 1–5 mgm. per cent uric acid.

Results. A. *The blood allantoin concentration of normal rats.* A distribution chart constructed from the results of 113 analyses of the blood allantoin of 72 rats is presented in figure 1. The mean value was found to be 2.1 mgm. per cent and the median value, 1.8 mgm. per cent. Eighty-eight per cent of the allantoin values lay in the range from 1 to 2.5 mgm. per cent. The extreme range varied from 0.6 to 3.2 mgm. per cent.

The average blood uric acid of the group was found to be 1.5 mgm. per cent (range: 0.5 to 3.4 mgm. per cent).

B. *The blood allantoin and uric acid concentration after nephrectomy.* Blood allantoin concentration rose immediately, sharply, and steadily in the 42 rats after bilateral nephrectomy, as shown in figure 2. Its accumulation in blood resulted in the following average concentrations: Pre-operative, 2.1 mgm. per cent; 6 hours after nephrectomy, 11.3 mgm. per cent; 15 hours, 23 mgm. per cent; 24 hours, 32 mgm. per cent; 48 hours, 38 mgm. per cent; and 72 hours, 50 mgm. per cent.

Blood uric acid, however, remained substantially unchanged (see fig. 2).

C. *The blood allantoin and uric acid concentration after hepatectomy.* Eight rats were hepatectomized. Blood uric acid increased rapidly following hepatectomy whereas blood allantoin concentration either remained the same or fell. The average pre-operative blood uric acid was 1.6 mgm. per cent, rising to 14 mgm. per cent 6 hours after hepatectomy.

Three of these animals were first subjected to a nephrectomy. As figure 3 indicates, the average blood allantoin in these animals rose from 1.9 mgm. per cent before nephrectomy to 24 mgm. per cent 15 hours after nephrectomy. The average blood uric acid concentration, however, showed little change in that it was 1.0 mgm. per cent before and 1.2 mgm. per cent 15 hours after nephrectomy. However, as soon as hepatectomy had been performed on these same rats, the blood allantoin concentration ceased to rise (see fig. 3). On the other hand, the blood uric acid concentration rose from 1.2 mgm. per cent before, to 13 mgm. per cent 6 hours after hepatectomy.

Discussion. In the present study the blood allantoin concentration of normal rats was determined. As far as we know this is the first report of the blood allantoin concentration in a large series of this species maintained on a relatively normal diet. Christman, Foster, and Esterer (3), however, have reported the blood allantoin in 13 rats existing on either a high or a low casein diet. The average concentration of their two groups was 1.22 mgm. per cent, a value considerably lower than the mean concentration of 2.1 mgm. per cent and median of 1.8 mgm. per cent found in our series. Our animals, of course, were receiving exogenous purine in their diet.

The rapid rise of blood allantoin and the absence of any increase in blood uric

acid following nephrectomy furnished suggestive evidence that the conversion of uric acid to allantoin was responsible for the increase in blood allantoin following nephrectomy. Previous investigators (1, 13) have shown that no significant rise in blood uric acid occurs in the dog and rabbit after nephrectomy. However, the blood allantoin had not been determined in such experiments although it was tacitly assumed to rise. Our results indicate that such a rise actually does occur.

The rapid rise of blood uric acid after hepatectomy has been reported previously without concomitant determinations of blood allantoin. Our studies also showed a rise in blood uric acid after hepatectomy. Moreover, it was found that blood allantoin under such circumstances failed to rise. In three animals subjected first to nephrectomy and then to hepatectomy, blood allantoin was found to increase in concentration until hepatectomy had been performed, after which time, it failed to increase. On the other hand, blood uric acid did not increase until hepatectomy had been done. These procedures clearly indicated that the liver of the rat functioned to convert blood uric acid into blood allantoin. Furthermore, when the blood uric acid concentration 6 hours after hepatectomy (average: 14 mgm. per cent or 4.66 mgm. of nitrogen per cent) was compared with the blood allantoin concentration 6 hours after bilateral nephrectomy (11 mgm. per cent or 3.89 mgm. of nitrogen per cent) it was found that a difference of only 16 per cent existed between the blood purine nitrogen concentrations of the two series of animals. When the extensive surgery sustained by rats subjected to the hepatectomy-viscerectomy is considered, this difference of 16 per cent is of questionable significance. It seems likely then in the normal rat, that the uric acid not excreted in the urine is converted entirely into allantoin and that this conversion is accomplished chiefly, if not exclusively, by the liver.

The presence of uricase in the rat liver has been reported by Lan (8), by Klemperer *et al.* (7) and by Truszkowski and Goldmanowna (14). The latter investigators also reported a small amount of uricase present in rat kidney. Five of our 8 hepatectomized animals had intact, but anuric kidneys, yet blood uric acid accumulated without any rise in blood allantoin. It is evident that if kidney uricase were present, it was ineffective in these hepatectomized rats.

SUMMARY

1. The median blood allantoin concentration of the adult male Wistar rat on a Purina chow diet was found to be 1.8 mgm. per cent.

2. Concomitant determinations of blood uric acid and allantoin before and after nephrectomy and hepatectomy suggested that rat blood uric acid is converted entirely to blood allantoin and that this conversion is accomplished chiefly, if not exclusively, by the liver.

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THE EFFECT OF INSULIN UPON THE LEVEL OF BLOOD AMINO ACIDS IN THE EVISCERATED RAT AS RELATED TO THE LEVEL OF BLOOD GLUCOSE

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Frame and Russell (1) found that the administration of insulin to the "functionally" eviscerated rat caused a significant decrease in the rate and extent of rise in the blood amino acid content. The present study extends the observations upon the eviscerated rat and supports the general conclusions of Frame and Russell by showing that the effect of insulin upon the level of blood amino acids is determined by the dose of insulin *per se* and is not modified significantly by wide changes in the level of blood glucose caused by the intravenous administration of different glucose loads at each insulin dose.

METHODS. Male rats of the Sprague-Dawley strain were used in these experiments. The diet was Purina Dog Chow. When the rats reached a weight of 185 to 205 grams the inferior vena cava was ligated between the liver and the kidneys in order to cause the development of a collateral circulation. Asepsis was preserved in this operation. When the animals reached a weight of 250 ± 2 grams they were anesthetized (intraperitoneal injection of 18 mgm. of cyclopentenyl-allyl-barbituric acid sodium) and eviscerated by the procedure of Ingle and Griffith (2). All of the intra-abdominal organs were removed except the adrenals and the kidneys. The animals were not fasted. In these liverless animals one initial dose of the barbiturate was sufficient to maintain effective anesthesia throughout the experiment.

Intravenous infusions of a 0.9 per cent sodium chloride solution with and without added glucose and crystalline zinc insulin (Lilly) were made by two continuous-injection machines which delivered fluid from each syringe at the rate of 20 cc. in 24 hours. Syringes of the Luer-Lok type were selected to deliver 20 cc. with a stroke of 65 mm. Two syringes were operated by one machine and 6 syringes by the other. Each was powered by a synchronous motor, and the reduction of motion was achieved by a precision-built system of gears so that an exact control of the rate of injection was maintained. The infusions were made into the saphenous vein of the right hind leg and were started within 3 minutes following the removal of the liver. The animals were secured in a supine position on an animal board and were enclosed in a cabinet having a constant temperature of 26.5 ±0.5 degrees C.

At the end of the infusion period blood was drained from the abdominal aorta by a cannula. Heparin was used as an anticoagulant. The analyses of blood glucose were by the method of Miller and Van Slyke (3). This procedure measures small amounts of non-fermentable reducing substances which collect in the blood of eviscerated rats. Whole blood was pooled from 2 to 4 animals,

and its amino acid content was determined by the ninhydrin-carbon dioxide method of Hamilton and Van Slyke (4).

Experiments and results. In experiment 1 (fig. 1) the values for glucose and amino acids of whole blood were determined at the end of 24 hours following evisceration. The levels of insulin dosage were 0.0, 0.25, 0.5, 1.0, 2.0, 4.0 and 16.0 units per rat per 24 hours. At each insulin dose, 3 different glucose loads were tested with 8 to 10 rats per load. The highest load caused hyperglycemia, the median load maintained a near normal level of blood glucose, and the smallest load permitted the development of hypoglycemia. The final level of blood amino acids was inversely proportional to the size of insulin dose and was not related to the glucose load or to the final level of blood glucose in these experiments. Ten rats which had recovered from ligation of the vena cava but were

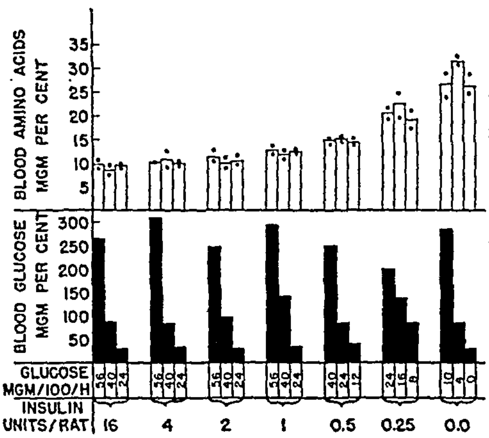

Fig. 1

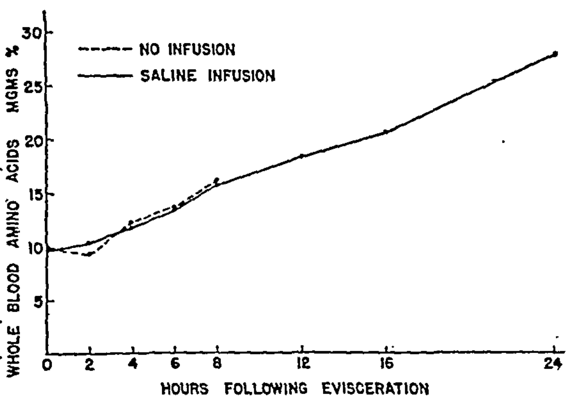

Fig. 2

Fig. 1. Level of amino acids in whole blood in relationship to insulin dose, glucose load and blood glucose level, 24 hours following evisceration. Averages and range of values.

Fig. 2. Changes in whole blood amino acids in eviscerated rats not given insulin or glucose. Averages.

not eviscerated were found to have an average level of 9.9 mgm. of amino acids per cent of whole blood. Eviscerated rats not given insulin had an average level of 28.4 mgm. per cent at the end of 24 hours, whereas the administration of large amounts of insulin to similar animals completely prevented the rise in amino acids.

In experiment 2 (fig. 2) the concentration of amino acids in whole blood was determined at the end of 2, 4, 6, 8, 12, 16 and 24 hours of continuous infusion of 0.9 per cent sodium chloride solution. The rate of increase in blood amino acids was much less than was reported by Frame and Russell (1) who found an average value of 43.3 mgm. per cent at the end of $5\frac{1}{2}$ to $6\frac{1}{2}$ hours as compared to the average value of 13.5 mgm. per cent at the end of 6 hours in the present experiment. It was considered possible that the intravenous administration of fluid in these experiments may have masked a true rise in blood amino acids by causing hemodilution. Accordingly, an additional series of blood samples was taken at

2, 4, 6 and 8 hours following evisceration in animals which were not given any fluid. The rate of increase in blood amino acids (fig. 2) was essentially the same as shown by similar animals given intravenous fluids. Kidney function was well maintained for several hours in the eviscerated animals of these experiments, and there was no significant change in the cell volume of the blood in those animals which received intravenous fluid and those which did not.

Experiment 3 was a further attempt to identify the factors responsible for the quantitative differences in values for amino acids observed in this study as compared to the data of Frame and Russell which were derived from animals having a "functional" evisceration and which were fasted for 24 hours prior to operation. Accordingly, the concentration of amino acids in whole blood was determined 6 hours following the evisceration of rats that had been fasted 24 hours prior to operation. Eight rats were eviscerated by the technique used in the above experiments and 8 rats were subjected to "functional" evisceration by the technique of Frame and Russell. These animals were not subjected to a preliminary ligation of the inferior vena cava; the gut was removed but the liver remained *in situ* after its arterial and portal vessels were tied. The rate of rise in blood amino acids was not greatly increased by the 24-hour fast and was not greater in the "functionally" eviscerated animals. The mean value at the end of 6 hours following "functional" evisceration was 14.7 mgm. per cent of whole blood as compared to 15.4 mgm. per cent for the completely eviscerated group.

Discussion. The data of these experiments are consistent with the conclusions of Frame and Russell (1) that the rise in blood amino acids which follows evisceration in the rat is suppressed by the administration of insulin and that this effect is not related to the blood sugar level. We have more fully explored the dose-response effect of insulin at high and low levels of glucose load and at high and low levels of blood glucose than was done by Frame and Russell. The level of blood amino acids was responsive to the amount of insulin *per se* and was not determined by the level of blood glucose. It is not possible to conclude whether the change in the level of blood amino acids represents a direct effect of insulin upon protein metabolism or whether it is an indirect effect which is secondary to the action of insulin upon the metabolism of carbohydrate. Frame and Russell have reviewed the other studies showing an effect of insulin upon nitrogen metabolism. More recently Friedberg and Greenberg (5) have shown that insulin depresses the level of amino acids in the blood of intact rats.

Although the general results of this study support the conclusions of Frame and Russell, there are significant quantitative differences in the values reported for the levels of amino acids in whole blood. They reported an average initial value of 15.1 mgm. per cent which increased to 43.3 by $5\frac{1}{2}$ to $6\frac{1}{2}$ hours and 65.0 by 8 hours. We found an average initial value of 9.9 which increased to 13.5 at 6 hours and 15.8 at 8 hours. Frame and Russell found only a partial suppression of the rise in amino acids by insulin, whereas we found complete suppression with insulin. According to experiments 2 and 3 our lower values were not due to the intravenous infusion of fluids, nor to our failure to fast the animals

for 24 hours nor to the difference in operative technique. The methods of chemical analysis of blood for amino acid content were different, and it is possible that the differences in results were due to differences in the specificity of these methods.

The use of a constant temperature chamber in these experiments upon anesthetized, eviscerated rats has tended to stabilize the effect of insulin upon the level of blood glucose as compared to the lower and more variable results reported earlier (6, 7). The effect of insulin upon glucose tolerance is increased by a rise in temperature of as little as 1 degree C. The temperature of the anesthetized, eviscerated rat falls rapidly so that within 2 to 3 hours following operation it is stabilized at 1 or 2 degrees above the temperature of the surrounding air. We had anticipated that the maintenance of a higher environmental temperature would improve the viability of these animals. To the contrary, an increase in environmental temperature above 28 degrees C. is followed by a marked reduction in survival time. Twenty-six-and-one-half degrees C. has been selected as standard for our experiments.

SUMMARY

Eviscerated male rats were given continuous intravenous infusions of different concentrations of glucose and insulin over a period of 24 hours. In rats not given insulin the amino acid concentration of whole blood increased from a normal average of 9.9 mgm. per cent to 28.4 mgm. per cent at the end of 24 hours. The administration of optimal amounts of insulin completely suppressed this rise.

The quantitative effect of insulin in suppressing the rise of blood amino acids was proportional to the dose and was not determined by the glucose load or by the level of blood glucose.

The rate of rise in blood amino acids in rats not given glucose or insulin was steady during the 24-hour period and was not changed significantly by the intravenous administration of fluids during a period of 8 hours.

The rate of rise in blood amino acids for 6 hours was increased only slightly by a 24-hour fast and was practically identical to that of similar animals subjected to "functional" evisceration instead of complete evisceration.

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THE THYROID SECRETION RATE OF GROWING AND MATURE MICE[1]

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Few investigators who have administered thyroid material to animals have determined dosage levels on the basis of the animal's own thyroid secretion rate. It is generally believed that the amount of thyroid hormone in the blood controls the secretion of the thyrotrophic hormone by the anterior pituitary gland. In order to produce a hyperthyroid condition more thyroid material must be given to an animal than its own thyroid gland secretes because of the depressing effect on thyrotrophic hormone secretion by added amounts of thyroid material. The present work deals with the normal thyroid secretion rate of growing and mature male and female mice determined through the administration of thyroxine and the feeding of thiouracil simultaneously. The thiouracil prevents the formation of endogenous thyroid hormone with a resulting increased secretion of thyrotrophic hormone. The thyroid gland responds by enlargement, and it is only by increasing the blood level of thyroid hormone to normal that the thyroid gland may be maintained at a normal size.

Dempsey and Astwood (3) first suggested a thyroid secretion assay by determining the amount of thyroxine needed to maintain normal thyroid size when administered concurrently with a goitrogenic drug. This assay has been used in the study of thyroid secretion rates in the rat (1, 3, 5, 7–10), and in the chicken, goat, and bovine (13).

PROCEDURE. Albino mice were purchased from a commercial breeder[2] and fed a ration mixed at this station which has proved satisfactory for growth, gestation, and lactation. Over the two week assay period the experimental animals were fed ad libitum with 0.2 per cent thiouracil[3] in the ration and injected subcutaneously daily with varying levels of d,l-thyroxine dissolved in the form of the di-sodium salt. Animals were weighed daily and injected in proportion to body weight. On the fifteenth day animals were sacrificed, the thyroids removed and weighed on a precision balance to the nearest 0.05 mgm.

Thyroid secretion rates were measured during the summer and winter seasons. During the summer there was no control of environmental temperature, the range being 85 to 92°F., with an average of 87°. In the winter thermostatic control of our animal room allowed a temperature range of 78 to 82°F., with an average temperature of 80°.

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[2] Ed. Schwing, Harrison, Ohio.

[3] We wish to thank Dr. Mark Welsh of Lederle Laboratories, Pearl River, N. Y., for the thiouracil used in this study.

The values reported here are in terms of injected d,l-thyroxine which has one-half the potency of l-thyroxine (11), the form in which thyroxine is active in thyroglobulin.

RESULTS. Young mice, at three to five weeks of age, were fed thiouracil and injected with thyroxine simultaneously. The mice were not segregated according to sex because of the small numbers in the groups and also because no differences were observed in the responses to treatment by the males and females. With the environmental temperature averaging 80°F., the young mice secreted the equivalent of 9.2 μg. of d,l-thyroxine per 100 grams body weight per day (table 1, fig. 1). Inasmuch as these animals were growing, they served to illustrate an interesting point in connection with the thyroid secretion assay method used in this work. With the animal's own thyroid secretion inactivated by the feeding of thiouracil, the only source of thyroid hormone would be the amount that was being injected. It would follow, therefore, that the amount

TABLE 1

Thyroid secretion rate

Growing mice, 3–5 weeks of age

THIOURACIL IN FEED	d,l THYROXINE INJECTED/100 GM. BW./DAY	NO. OF MICE	BODY* WT.	THYROID* WT.	THY. WT.*/100 GM. BODY WT.
%	μg.		*grams*	*mgm.*	*mgm.*
None	None	11	15.6±1.90	2.07±0.13	13.3± 1.09
0.2	None	9	12.1±0.33	10.50±1.28	86.8±10.23
0.2	2.5	9	11.2±0.36	8.64±0.91	77.9± 4.76
0.2	5.0	11	15.3±0.43	6.05±0.96	39.5± 6.33
0.2	10.0	8	14.8±0.09	1.20±0.29	8.1± 1.28

Estimated thyroid secretion rate/day; 9.2 μg. per 100 gram body weight.
* All means given with standard errors.

of thyroxine required to maintain the thyroid gland at normal size would also be enough to cause normal growth gains. This was found to be the case (table 1, fig. 1). The animals which received 10 μg. of d,l-thyroxine per 100 grams body weight per day gained 85 per cent of their initial body weight as compared to a gain of 93 per cent by the controls. The differences in the body weights between the group injected with 10 μg. of thyroxine and the controls were not significant, according to an analysis of variance (14), either at the beginning or the end of the assay period.

Mature mice, assayed for thyroid secretion rate during the winter months when temperature was held at about 80°F., secreted less thyroid hormone than did the young mice on a 100 gram body weight basis under the same conditions. Mature female mice secreted the equivalent of 5.5 μg. of d,l-thyroxine per 100 grams body weight per day, or about one-half of what young mice produce, and mature male mice secreted the equivalent of 2.4 μg. of d,l-thyroxine per 100 grams body weight per day, or about one-fourth of the amount produced by young mice (table 2, fig. 2). It follows then that the thyroid secretion rate of

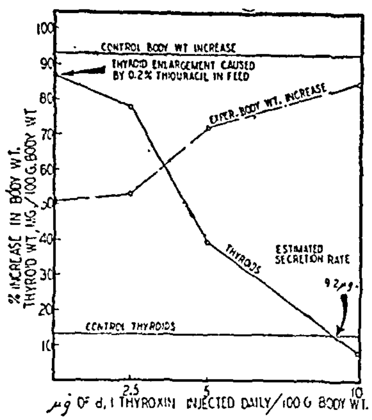

Fig. 1

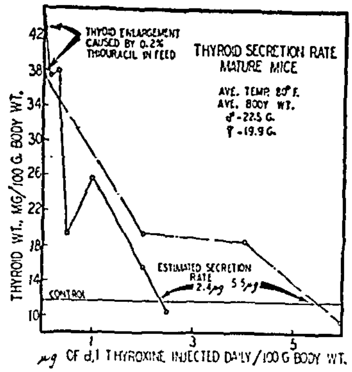

Fig. 2

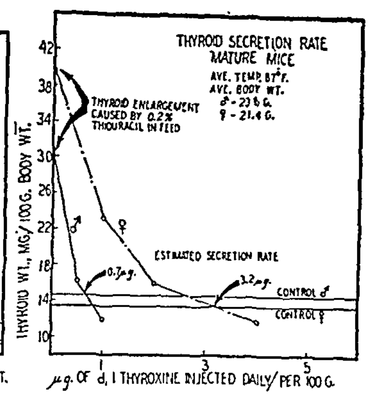

Fig. 3

Fig. 1. Thyroid weights and percentage body weight increases of growing mice, 3 to 5 weeks of age, injected for 14 days with thyroxine during the simultaneous feeding of thiouracil.

Fig. 2. Thyroid weights of mature mice kept at 80°F., and injected for 14 days with thyroxine during the simultaneous feeding of thiouracil. Solid line, males; broken line, females.

Fig. 3. Thyroid weights of mature mice kept at 87°F., and injected for 14 days with thyroxine during the simultaneous feeding of thiouracil.

TABLE 2

Thyroid secretion rate-80°F.

THIOURACIL IN FEED	d,l THYROXINE INJECTED/100 GM. BW./DAY	NO. OF MICE	BODY* WT.	THYROID* WT.	THY. WT.*/100 GM. BODY WT.
			Mature males		
%	µg.		*grams*	*mgm.*	*mgm.*
None	None	74	20.5±0.47	2.46±0.12	11.6±0.42
0.2	None	38	22.9±0.39	9.72±0.50	42.8±1.72
0.2	0.125	19	22.3±0.45	8.52±0.67	37.4±2.54
0.2	0.25	19	23.6±0.49	8.95±0.68	37.9±2.57
0.2	0.5	5	21.2±1.38	4.09±0.84	19.3±2.75
0.2	1.0	8	22.8±1.29	5.86±0.81	25.7±3.20
0.2	2.0	8	21.9±0.65	3.40±0.49	15.5±2.09
0.2	2.5	10	22.1±0.50	2.32±0.39	10.5±1.88
Estimated thyroid secretion rate/day; 2.4 µg./100 gram body weight; 0.54 µg./mouse; ave. body wt. 22.5 gram.					
			Mature females		
None	None	24	19.9±0.29	2.44±0.14	11.7±0.62
0.2	None	10	20.1±0.63	7.17±0.60	37.3±4.17
0.2	2.0	10	19.8±0.64	3.85±0.48	19.4±2.29
0.2	4.0	10	19.5±0.37	3.60±0.72	18.5±3.35
0.2	6.0	10	20.1±0.46	1.84±0.19	9.2±0.87

Estimated thyroid secretion rate/day; 5.5 µg./100 gram body weight; 1.09 µg./mouse; ave. body weight 19.9 grams.

* All means given with standard errors.

mice declines with age. Another point of interest is that whereas there was no difference between male and female thyroid secretion rate in young mice, a difference between sexes did show up in the mature mice. Females secreted somewhat more than twice as much thyroid hormone as did the males.

In studying the thyroid secretion rate during the summer months, the temperature ranged from 85 to 92°F. with a mean of 87°. The higher temperature caused the thyroid secretion rate to decline in mature animals as compared to studies made at 80°F. Male mice secreted the equivalent of 0.7 μg. of d.l-thyroxine per 100 grams body weight per day, or about one-fourth as much as at 80°F., and the females 3.2 μg., somewhat more than half of the amount

TABLE 3

Thyroid secretion rate-87°F.

THIOURACIL IN FEED	d, l THYROXINE INJECTED/100 GM. BW./DAY	NO. OF MICE	BODY* WT.	THYROID* WT.	THY. WT.*/100 GM. BODY WT.
Mature males					
%	μg.		grams	mgm.	mgm.
None	None	9	26.1±1.30	3.84±0.44	14.7±1.49
0.2	None	7	24.2±0.66	7.33±1.02	30.3±3.78
0.2	0.5	10	21.6±0.40	3.53±0.37	16.3±1.67
0.2	1.0	11	23.1±0.96	2.78±0.24	12.0±1.12
Estimated thyroid secretion rate/day; 0.7 μg./100 gram body weight; 0.17 μg./mouse; ave. body weight 23.6 grams.					
Mature females					
None	None	9	20.6±0.56	2.81±0.18	13.6±0.78
0.2	None	10	21.5±0.52	8.53±1.15	39.7±5.79
0.2	1.0	9	21.5±1.00	5.00±0.62	23.2±2.66
0.2	2.0	10	22.1±0.27	3.56±0.34	16.1±1.46
0.2	4.0	9	21.2±0.62	2.52±0.21	11.9±0.83
Estimated thyroid secretion rate/day; 3.2 μg./100 gram body weight; 0.68 μg./mouse; ave. body weight-21.4 grams.					

* All means given with their standard errors.

secreted at the lower temperature (5) (table 3, fig. 3). These results show that there is a sex difference also at 87°F., the females secreting almost five times as much thyroid hormone as the males on the basis of 100 grams' body weight.

Because of the differences observed in the thyroid secretion rate between young and mature mice, between mature males and females, and because of the effect of temperature on thyroid secretion rate, the investigator must regulate thyroid stimulation with regard to the animal's own response to physiological and environmental differences. At 87°F., for example, many male mice died following a dosage level that was only enough to maintain a normal thyroid hormone level in the blood of the females at the same temperature. If one wished to stimulate growing mice and gave twice the amount of hormone secreted

by the mature male at 80°F., or about 5 μg., he would not stimulate the growing mice but simply complement the thyroid secretion by the amount added inasmuch the growing animal itself is already producing the equivalent of 9.2 μg. of d,l-thyroxine per 100 grams body weight per day. Any amounts of thyroid material given to animals up to and including their normal thyroid secretion rate simply cause a reduction in thyrotrophic hormone secretion by the anterior pituitary gland with a resultant decrease in thyroid hormone production by the animal's own thyroid. The net result then would be the same as if no hormone had been administered.

DISCUSSION. In agreement with the work reported here in the mouse, other workers have noted a decline in thyroid secretion per 100 grams of body weight with the increase in size of the growing animal. Schultze and Turner (13) reported this in the chicken and Monroe and Turner (8) in the rat.

The difference in sex response to thyroxine injections has already been observed in the mouse by Schoeller and Gehrke (12). They reported that equal amounts of thyroxine injected on a body weight basis stimulated greater carbon dioxide production in males than in females. Schultze and Turner (13) noted small but consistently greater thyroid secretion rates in growing male White Plymouth Rock chickens as compared to females. Monroe and Turner (8) reported that young female rats secreted more thyroid hormone than males, but that as the animals grew older the sex difference was reversed. Schultze and Turner (13) fed the dimethyl ether of diethylstilbestrol to Barred Rock pullets and found that their thyroid secretion rate was not significantly different. The same authors also noted that castration lowered the thyroid secretion rate of young Plymouth Rock chickens.

The differences observed in thyroid secretion rates at different temperatures agree with earlier observations on the rat. The curves obtained on the rat by Dempsey and Astwood (3) agree with ours in that at cooler temperatures both show a steeper response slope to thyroxine injections. It is also of interest to correlate our observations on temperature with those of Herrington (4) who studied the basal metabolism of the mouse. He found the "zone of thermoneutrality", defined by Brody (2) as "the temperature at which heat loss from the body is equal to the minimum heat production", to be between 85–92°F. On this basis, the mice kept at 80°F. would increase their basal metabolic rate in order to compensate for heat losses from the body. These values are correlated with the present work in which the thyroid secretion rate increased in both sexes at 80°F., outside of the zone of thermal neutrality.

It is of interest at this point to correlate the thyroid secretion rate of mice with that of other species studied in these laboratories (table 4). On the basis of 100 grams of body weight, the male mouse ranks second to the male rat, the rat producing about one and one-half times as much hormone as the mouse. With the females and growing animals, however, the mouse produces more thyroid hormone than the other species studied. Thus female mice secrete about three and one-half times as much thyroid hormone as White Plymouth Rock chickens, or twice as much as rats on a body weight basis. Growing

TABLE 4

Thyroid secretion rate of several species

ANIMAL	BODY WEIGHT	THYROID SECRETION RATE, μg. d,l THYROXINE EQUIV./DAY	DAILY THYROID SECRETION RATE, μg. d,l THYROXINE EQUIV./100 GM. B.W./DAY	TEMPERATURE
		μg.	μg.	°F.
White Leghorn cockerels (13)	343.0 gram	7.55	2.20	82–86
	1536.0 gram	25.00	1.36	82–86
White Leghorn laying hens (15)	1965.8 gram	11.85	0.60	January
White Plymouth Rock cockerels (13)	410.0 gram	8.10	1.98	82–86
	1502.0 gram	23.00	1.53	82–86
White Plymouth Rock pullets (13)	360.0 gram	8.75	2.43	82–86
	1637.0 gram	26.00	1.59	82–86
Growing goats (13)				
males and females	10.0 kgm.	180.00	1.80	50–80 probable range
females	20.4 kgm.	640.00	3.13	50–80 probable range
females	34.5 kgm.	930.00	2.70	50–80 probable range
Lactating goats (13)				
(milk yield 1.8 lbs./day)	43.5 kgm.	1000.00	2.29	50–80 probable range
(milk yield 2.6 lbs./day)	43.5 kgm.	1425.00	3.44	50–80 probable range
Calf (13)	72.6 kgm.	1500.00	2.06	50–80 probable range
Cow (lactating) (13)	454.0 kgm.	10,000.00	2.20	50–80 probable range
Male rats (8)	85.2 gram	3.10	3.64	78
	275.7 gram	9.54	3.46	78
Female rats (8)	81.1 gram	3.75	4.63	78
	266.2 gram	7.51	2.82	78
Pregnant rats (8)	190.8 gram	5.59	2.93	78
Lactating rats (8)	209.1 gram	6.48	3.10	78
Growing mice	14.8 gram	1.36	9.20	80
Mature male mice	22.5 gram	0.54	2.40	80
	23.6 gram	0.17	0.70	87
Mature female mice	19.9 gram	1.09	5.50	80
	21.4 gram	0.68	3.20	87

mice secrete four times as much thyroid hormone as the chick and twice as much as the growing female rat.

SUMMARY

The thyroid glands of young mice averaging 14.8 grams at five weeks of age produced the equivalent of 9.2 μg. of d,l-thyroxine per 100 grams body weight per day. No sex differences were observed.

Mature male mice kept at an average of 80°F. produced the equivalent of 2.4 μg. of d,l-thyroxine per 100 grams body weight per day, and mature females secreted 5.5 μg.

At higher temperatures averaging 87°F., mature males secreted the equivalent of 0.7 μg. of d,l-thyroxine per 100 grams body weight per day and the females, 3.2 μg.

From the results of these studies it has been shown that physiological and environmental conditions affect the thyroid secretion rate in mice.

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ISCHEMIC COMPRESSION SHOCK; INFLUENCE OF BODY TEMPERATURE AND OF TEMPERATURE OF TRAUMATIZED TISSUES[1]

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In other publications (Green and Bergeron, 1; Bobb and Green, 2) it was established that shock due to compression trauma increases in severity as the environmental temperature is elevated, and decreases within limits as the environmental temperature is lowered. The present study was designed to determine the relative part played by the environmental temperature through its influence (a) on the body temperature and (b) on the temperature of the traumatized tissues.

METHODS. Random mongrel dogs were anesthetized by a subcutaneous injection of 2 mgm. per kgm. of morphine followed in 1 to 2 hours by an intravenous injection of 20 mgm. per kgm. of sodium pentobarbital[2]. While some of the animals received additional sodium pentobarbital during the first part of the period of compression, none of them received any medication after the first 4 hours. Food was withheld for 20 hours preceding and for 30 hours following the beginning of the experiment. Water was withheld for 30 hours, beginning with the onset of anesthesia.

All animals were traumatized by wrapping both hind legs with a tightly drawn continuous spiral of rubber tubing from the ankle to the groin (3). This procedure produced both crushing and ischemia of the muscles of the hind legs. As soon as the compression tubes were in place the animals were placed on their side on animal boards in a constant temperature room. They remained in the room for 48 hours or until death.

The period of compression was 6 hours and during this time the temperature of the hind legs was controlled by circulating water through a second set of rubber tubes wrapped outside the compression tubes. The body temperatre was controlled by varying the temperature of the constant temperature room. After removal of the compression tubes, the animals were allowed to move about freely in cages in the constant temperature room. The rectal temperature, the temperature of the traumatized tissues, and the room temperature were recorded by means of iron constantan needle thermocouples, using a Leeds and Northrup micromax recorder.

The volume of each hind leg was measured by immersing the leg in a tall, cylindrical chamber filled with water before applying the compression tubes and

[1] Aided by Grant no. 543 from the Council on Pharmacy and Chemistry of the American Medical Association.

[2] The sodium pentobarbital used in these experiments was supplied through the courtesy of the Premo Pharmaceutical Laboratories, 443 Broadway, New York City.

again after death, or in the case of survival, after 48 hours. The difference in these two readings is recorded as the local edema, the figure in the tables being the sum of the edema in the two legs.

RESULTS. Three groups were studied as indicated in table 1. Group I, the control group, consisted of 9 dogs. The average environmental temperature was 32.6°C., and the hind limbs were maintained at approximately 38°C. Group II consisted of 10 dogs, studied at a lowered environmental temperature (approximately 20°C.) but with the temperarure of the traumatized tissues approximately the same as the control group. Group III consisted of 8 dogs for which the average environmental temperature was approximately the same as the control group (32.8°C.), but in which the traumatized limbs were maintained at a lowered temperature (average 24.1°C.).

Table 1 records the results of the experiment. There were no indefinite survivals, defined as those animals that lived for longer than 48 hours, in group I; the average time of survival was 6.2 hours. Group II had a 10 per cent indefinite survival. The remaining dogs' average survival time was twice that of the control group. Group III had a 25 per cent indefinite survival and the average for the remaining animals was 2.4 times that of the control group.

TABLE 1

GROUP	HIND LEG TEMP. (°C.)		ENVIRON. TEMP. (°C.)		RECTAL TEMP. (°C.)		NO. DOGS	NO. SURV.	SURV. (HRS.)		EDEMA (ML./KGM.)	
	Range	Av.	Range	Av.	Range	Av.			Range	Av.	Range	Av.
I	38.5–39.0	37.8	30.0–35.0	32.6	37.0–42.0	40.3	9	0	2.5–17.8	6.2	8.9–36.8	22.3
II	36.0–38.5	37.5	19.0–20.0	19.7	30.5–37.0	34.1	10	1	2.0–32.0	12.4	15.4–48.2	27.1
III	21.5–26.5	24.1	32.5–33.0	32.8	34.5–40.0	37.9	8	2	6.1–24.6	14.9	−0.2–39.1	15.0

The local edema in the control group averaged 22.3; in group II, 27.1; and in group III, 15.0 ml./kgm. The minus value in one case represented a decrease in limb volume possibly because the amount of edema produced was not sufficient to overcome the compression of the tissues caused by the wrappings.

DISCUSSION. Table 2 is the result of a statistical analysis of the data presented in table 1. The t test and the Fisher tables of probability for this test were used. Comparison of the survival times of groups I and II shows a probability slightly above the critical value of 5 per cent that such a difference might be exceeded by chance. We, nevertheless, believe this difference to be statistically significant, particularly in view of the one indefinite survival in group II. The computation of the t value did not include this dog.

Comparison of the survival times of the animals in group I with those of the animals of group III which did not survive shows that the probability of obtaining a difference greater than this by chance is between 1 and 2 per cent. This indicates a high degree of significance, particularly in view of the 2 additional animals which survived indefinitely.

Comparison of the amounts of edema in groups I and II and I and III shows that the differences in average edema are not significant.

These experiments confirm the earlier studies in which it was demonstrated that placing the whole animals in a cool environment increased the chances of indefinite survival and prolonged the hours of survival of those that died and shows that the earlier results were in all likelihood due to the combined effect of the cool environment on the body of the animal, as well as on the traumatized tissues.

It is not possible, on the basis of these results, to give any explanation as to the mechanisms involved in the favorable response to cooling. It is quite probable that the greater survival of group II, as compared with group I, may have been due to diminishing the amount of loss of water from the body by evaporation, to facilitating the maintenance of cutaneous-vasoconstriction, and/or to diminishing the metabolic demands on the circulation. In previously published experiments it was shown that the metabolic activity of dogs is decreased by approximately 10 per cent for each degree lowering of body temperature (3). During the period after release of compression, the average rectal temperature in group II was approximately 7 degrees less than that of group I. On this basis it might be postulated that the metabolic demands in group II would be approximately 50 per cent of those in group I. A part of the greater survival might be attributed to this. The edema in the legs was certainly not less and, if anything, was greater in group II than in group I. We may conclude that the animals did not survive longer because of lessening of the edema in the traumatized tissues and, in fact, survived better despite some tendency for greater local edema formation.

TABLE 2

Statistical treatment

	SURVIVAL (HRS.) GROUPS I AND II	SURVIVAL (HRS.) GROUPS I AND III	EDEMA (ML./KGM.) GROUPS I AND II	EDEMA (ML./KGM.) GROUPS I AND III
MEANS	I 6.16 (9)* II 12.36 (9)	I 6.16 (9) III 14.90 (6)	I 22.3 (7) II 27.1 (10)	I 22.3 (7) III 15.0 (8)
Diff. between the means = $(\bar{X}_1 - \bar{X}_2)$...	6.20	8.74	4.8	7.3
t†....................	1.89	2.69	0.99	1.16
Probability‡............	>0.05; <0.10	>0.01; <0.02	>0.3; <0.4	>0.2; <0.3

* Figures in parentheses are the number of animals in each group.

$$\dagger t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{\Sigma(X_1 - \bar{X}_1)^2 + \Sigma(X_2 - \bar{X}_2)^2}{(N_1 - 1) + (N_2 - 1)}} \times \sqrt{\frac{1}{N_1} + \frac{1}{N_2}}}$$

‡ Probability = the likelihood that a difference numerically greater than this would be obtained by chance. Fisher's tables (5).

The greater survival in group III as compared with group I is probably due to the occurrence of a lesser degree of edema in the traumatized tissues. However, since the difference in edema in the two groups is not statistically significant, one may postulate that other factors also played a part. These experiments give no evidence as to what these other factors may be.

SUMMARY

A cool environment, which lowers the body temperature, and cooling of traumatized tissues while maintaining the body in a warm environmental temperature, both increase the survival of dogs subjected to ischemic compression trauma of their hind extremities. The improved survival cannot be explained on the basis of changes in local edema at the site of the trauma.

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EFFECT OF HEPARIN ON ISCHEMIC COMPRESSION SHOCK[1]

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In previous studies (1, 2) it was observed that dogs traumatized by a six hour period of ischemic compression showed shorter survival and more severe shock when anesthetized and cross-transfused with test dogs than when anesthetized alone for similar intervals of time. One possible factor in the increased severity is the heparin used to prevent coagulation of the blood during the cross-transfusion.

This paper is the report of a study designed to determine the effects of heparin upon ischemic compression shock (2). The study was initiated also because of the appearance in the current literature of reports that frostbite (3) and incipient gangrene (4), as well as other conditions often associated with trauma, were being treated by the use of heparin.

The low toxicity of heparin has been frequently described in the literature (5, 6), but apparently no observations have been made on animals in traumatic shock.

Methods. The procedures used in these experiments were essentially the same as those described in the paper by Lipton, Denison, and Green (8).

A cannula was placed in the external jugular vein of each dog to facilitate injection. Five hours after wrapping, 2.5 mgm./kgm. of heparin[2,3] in saline were injected intravenously into the test dog. This was followed by 0.5 mgm./kgm. every half-hour for 9 additional doses. The control dog received physiologic saline in volume equal in ml./kgm. to the volume of the heparin solution.

Results. The survival times and temperatures in the various experiments are tabulated in table 1. The survival time of all dogs kept at environmental temperatures above 30 degrees centigrade never exceeded 6 hours[4]. The average survival was 3.5 hours. No difference in survival was noticed between the heparinized and the non-heparinized animals.

Longer survivals were noted in the animals kept at lower environmental temperature, but again no differences were noted between the heparinized and non-heparinized animals.

Complete gross autopsies were performed on all animals. Submucosal hem-

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[2] The sodium pentobarbital used in these experiments was supplied through the courtesy of the Premo Pharmaceutical Laboratories, Inc., 443 Broadway, New York 13, N. Y.

[3] The heparin was kindly supplied by Lederle Laboratories Division, American Cyanamide Company, Pearl River, New York.

[4] A total of 22 dogs in this form of shock have been studied. In every case the dog survived six hours or less.

orrhages in the duodenum and upper jejunum; pulmonary edema; and increased interstitial fluid with blanching of the muscles of the wrapped extremities were seen in most of the dogs. No differences were noted between the heparinized and non-heparinized animals, nor between those dying at high or those at low environmental temperatures.

TABLE 1

DOG: H—HEPARINIZED, C—CONTROL	WEIGHT	AV. RECTAL TEMP.	AV. ROOM TEMP.	SURVIVAL TIME	DIFFERENCE IN SURVIVAL H—C
	kgm.	°C.	°C.	*hrs.*	*hrs.*
H—1	6.0	40.5	33.2	1.7	+0.2
C—1	3.9	38.4	33.2	1.5	
H—2	4.5	39.7	32.2	3.0	+0.2
C—2	6.6	37.0	32.2	2.8	
H—3	4.1	35.1	24.1	9.1	−3.6
C—3	4.8	35.2	24.1	12.7	
H—4	5.0	28.9	19.9	22.2	+15.0
C—4	6.1	32.2	19.9	7.2	
H—5	6.1	30.5	19.8	29.3	+11.5
C—5	5.4	29.2	19.7	17.8	
H—6	6.6	31.4	22.3	28.0	−1.0
C—6	7.0	36.1	22.3	29.0	
H—7	7.4	39.1	28.0	12.7	−15.8
C—7	4.4	38.3	27.8	28.5	
H—8	5.1	35.4	31.1	5.5	−0.5
C—8	6.6	34.0	31.1	6.0	
H—9	5.2	36.1	30.5	3.0	−0.3
C—9	7.7	37.0	30.5	3.3	
Average H..........	5.6	35.2	26.8	12.7	+0.6
C..........	5.8	35.3	26.8	12.1	

The differences in survival times were not statistically significant. In this case:

$$\frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{\Sigma(X_1 - \bar{X}_1)^2 + \Sigma(X_2 - \bar{X}_2)^2}{(N_1 - 1) + (N_2 - 1)} \times \left(\frac{1}{N_1} + \frac{1}{N_2}\right)}} = 0.12 \text{ which gives a probability} > 0.9 \text{ (7)}.$$

CONCLUSION

Heparin given in repeated doses over a $4\frac{1}{2}$ hour period in amounts sufficient to render the blood completely non-coagulable has no discernible effect upon the survival or upon the severity of the shock due to a preceding six hour period of ischemic compression of the hind extremities of anesthetized dogs.

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RÔLE OF THE KIDNEY IN RESISTANCE TO ISCHEMIC COMPRESSION SHOCK[1]

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In 1954 Goldblatt and his group demonstrated that hypertension was consistently produced by constricting the renal arteries of dogs (1). This hypertension was, at least initially, due to a vasopressor substance produced by the ischemic kidneys (Houssay and Fasciolo, 1937; Houssay and Taquini, 1938 (2)). It was further demonstrated by Goldblatt and Kahn (3) that constriction of the aorta above the kidneys produced hypertension which was also attributed to renal ischemia. The hypertension in either case appeared within a few minutes after the production of the ischemia (Enger, Linder and Sarre, 1938 (2)).

From this point it was apparently reasoned that the pressor substance is probably also produced when the mean arterial pressure falls markedly, as in shock, and the blood supply to all organs, including the kidneys, is decreased. Several groups of investigators sought and reported such a renal vasopressor substance in hemorrhagic shock (4, 5). Observing capillaries Zweifach and co-workers reported increased vasomotion, due to a substance traceable to the kidney (6) in certain phases of hemorrhagic (7) and tourniquet (8) shock. Using hemorrhagic shock it was observed by Hamilton and Collins and by Bahnson that in the dog (9, 10) and rat (11) the mean arterial pressure was better maintained and the survival period longer (rat) in the sham-operated than in nephrectomized animals. These findings led to rather widespread speculation on the importance of renal compensation in shock.

The experiments reported here were designed to test the importance of renal compensation in ischemic compression shock.

METHODS. Mongrel dogs, weighing 4 to 16 kgm., were anesthetized with an initial subcutaneous injection of 5 mgm./kgm. of morphine sulfate followed in a half-hour by an intravenous injection of 35 mgm./kgm. of sodium pentobarbital.[2] Subsequent small doses (3.5 mgm./kgm.) were given to keep the dog quiet. However, none was necessary after removal of the compression. They were traumatized by wrapping the hind legs very tightly with rubber tubing for 6 hours as previously described by Green, Dworkin, Antos and Bergeron (12).

The dogs were traumatized in pairs and placed side-by-side in a constant temperature room which was kept within range of 2 to 3 degrees for each pair of dogs. From other experiments (13) it was known that with environmental temperatures above 30°C. animals in shock as produced above could be expected to die in six

[1] Aided by a grant from the Ella Sachs Plotz Foundation.

[2] The sodium pentobarbital was kindly supplied by Premo Pharmaceutical Laboratories, Inc., 443 Broadway, New York 13, N. Y.

hours or less. Since mean arterial pressure readings were desired for the duration of the animal's life, temperature ranges between 28°C. and 33°C. were selected.

A cannula for recording the mean arterial pressure by mercury manometer was inserted into a carotid artery of each dog. One milliliter of a solution containing 50 mgm./ml. of heparin placed in the cannula and adjacent tube provided excellent control of clotting[3]. In the hour preceding removal of the compression, the kidneys were freed and vessels dissected in both dogs. Ligatures were

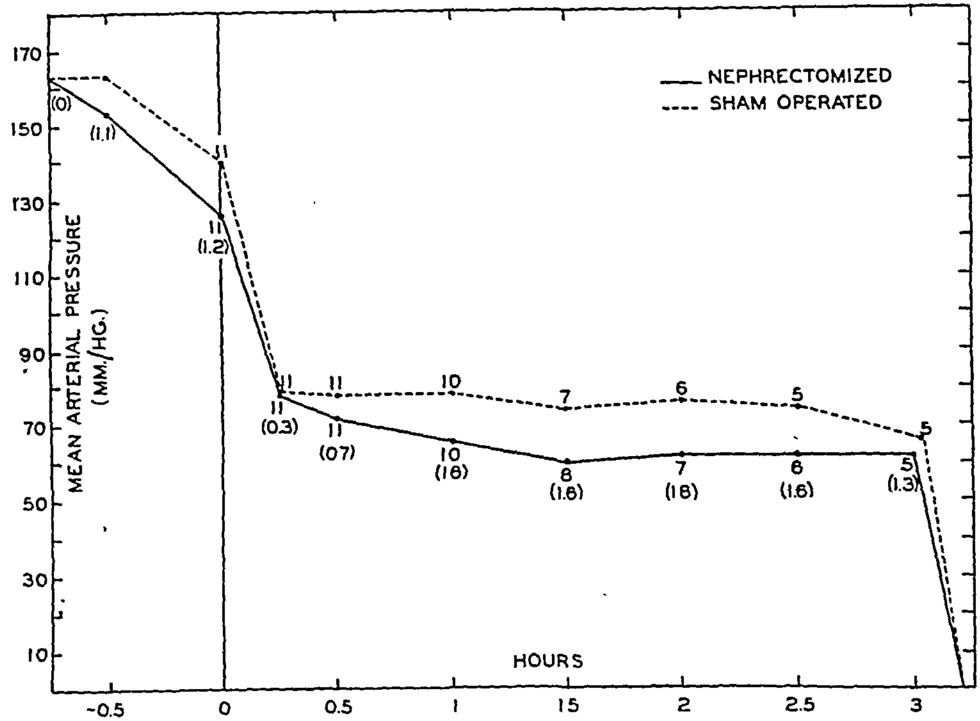

Fig. 1. Average mean arterial pressure of surviving dogs. Zero line represents the time at which compression was removed from the legs. The time to the left of the zero line represents the last ¾ hour of compression when the operative procedures were done. The numbers show the number of sham-operated and nephrectomized animals alive at each point. The figure in parentheses is the Fisher "t" value comparing the averages of the mean arterial pressures of the surviving nephrectomized animals with those of the surviving sham-operated dogs.

tightly tied around (or hemostats applied to) the renal pedicles in the test animals while in the sham-operated a loose ligature was placed about the pedicles.

Results. *Effect of nephrectomy on mean arterial pressure.* The average of the mean arterial pressures of the survivors of each group of dogs is plotted in figure 1. During the period of ischemic compression the mean arterial blood pressures were usually between 150 and 180 mm.; the arterial pressures of both groups began to fall in the hour prior to removal of compression presumably due to the trauma of nephrectomy, sham-operation and cannulation. The average fall in

[3] The heparin was supplied through the courtesy of Lederle Laboratories Division, Cyanamide Company, Pearl River, N. Y.

pressure in the first quarter hour on removal of the compression averaged 48 mm. for the eleven nephrectomized dogs and 60 mm. for the eleven sham-operated

TABLE 1

Comparison of the mean arterial pressure of pairs

PAIR NUMBER	TIME IN HOURS									
	¾	½	0	¼	½	+1	+1½	+2	+2½	+3
F1	−28	−18	−12	0	+2	+6	+36	+28	+12	+74*
F2	−10	−14	+6	−4	+6	+14	†	†	†	†
F3		−12	−4	−10	+6	+16	+20	+12	+24	+24
F4			0	−24	−20	+10	−4	0	†	†
F5	+56	+46	+28	+8	0	+4	−54*	†	†	†
F6			+6	+8	+22	+38	+56	+56*	+54*	+46*
F7			+52	−10	−16	+22	−38	−50*	−50*	−50*
F8	+6	−18	+8	−6	−38	−92*	−80*	−84*	−76*	−70*
F9	−60	+26	+36	0	+4	−20	−56*	−64*	−71*	−75*
F10		+52	−23	0	+20	+46	+38	+34	+32	+14
F11	+16	+32	+82	+70	+80	+96*	+86*	+86*	+80*	+64*
All pairs										
Average	−3	+12	+14	+4	+6	+13	+0.2	+2	0	+3
σ	39.7	30.2	30.1	23.9	30.4	39.0	56.6	56.9	59.9	60.2
t‡	0.20	1.11	1.53	0.55	0.65	1.10	0.01	0.10	0.0	0.14
(deg. freedom)	(5)	(7)	(10)	(10)	(10)	(10)	(9)	(8)	(7)	(7)
Living pairs only										
Average	−3	+12	+14	+4	+6	+15	+18	+18	+23	−19
σ	39.7	30.2	30.1	23.9	30.4	19.3	34.1	15.5	10.0	7.1
t	0.20	1.11	1.53	0.55	0.65	2.32	1.27	2.32	3.98	3.72
(deg. freedom)	(5)	(7)	(10)	(10)	(10)	(8)	(5)	(3)	(2)	(1)
Probability						0.05	0.2–0.3	0.1	0.05–0.1	0.1–0.2

These figures are the mean arterial pressures of the nephrectomized animals subtracted from the mean arterial pressures of the sham-operated dogs.

* One of this pair was dead. Therefore, its mean arterial pressure was zero.

† Both of pair were dead.

‡ Calculated: $t = \dfrac{d \times \sqrt{N}}{\sigma}$; $\sigma = \sqrt{\dfrac{\Sigma(d - \bar{d})^2}{N - 1}}$; d = the figures in the above table; $\bar{d}$ = average difference.

dogs. In the period of shock after release of the compression tubes the surviving dogs of the sham-operated group had an average pressure sustained from 2 mm. to 14 mm. higher than the nephrectomized. However, on statistical analysis,

using Fisher's "t" test, the differences are not significant at the 5 per cent level at any point.

Table 1 is an analysis of the mean arterial pressures of the individual pairs of dogs. Here there was only one statisitically significant average difference between the nephrectomized and sham-operated dogs (at 1 hr.).

Effect of nephrectomy on survival. Although there was great variability (−4.2 to +5.1 hrs.) in the difference of survival times of any pair of dogs the average difference is zero. The average survival for each group is exactly the same, 3.2 hours (table 2).

DISCUSSION. The results of these experiments appear to conflict with the results obtained by various investigators cited previously. However, in this case ischemic compression shock was used, while Hamilton and Collins and Bahnson used hemorrhagic shock. It is possible that the difference in the

TABLE 2

Survival time in hours

PAIR	NEPHRECTOMIZED	SHAM-OPERATE	DIFFERENCE
F10	4.3	8.5	−4.2
F6	1.5	4.6	−3.1
F11	0.7	3.5	−2.8
F1	2.9	5.4	−2.5
F3	3.2	4.3	−1.1
F2	1.5	1.6	−0.1
F4	2.3	2.4	−0.1
F5	2.0	1.2	+0.8
F7	4.6	1.5	+3.1
F8	5.6	0.7	+4.9
F9	6.2	1.1	+5.1
Average............	3.2	3.2	0

average mean arterial pressures of the two groups of animals may conceivably be due to a renal vasopressor substance. The small degree of this difference may be attributable to the fact that shock produced by ischemic compression is more severe in some ways than that produced by hemorrhage. For example, with extensive damage to small vessels such as produced in traumatic compression, the arterial pressure, if sustaned at higher levels, may lead to greater fluid loss than in the animals with a lower arterial pressure.

CONCLUSION

These experiments suggest that, while the presence of the kidneys in traumatized animals may produce a slightly higher mean arterial pressure during the period of ischemic compression shock, the overall survival time is not changed.

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STUDIES ON THE TEMPERATURE CHARACTERISTICS, BLOOD FLOW AND ACTIVITY IN NORMAL AND DENERVATED LIMBS OF THE DOG[1]

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Skeletal muscle when deprived of its motor nerve supply exhibits certain functional characteristics which differ from those of normal muscle (1). The nature and degree of difference depend somewhat upon the particular muscle and the species under consideration. The functional changes include the constant presence of fibrillary activity, an increased sensitivity to potassium, adrenalin and acetylcholine, a change in the strength-duration characteristics of excitation and a contracture response to both dorsal root stimulation and intravenous injections of acetylcholine. Changes in blood flow, temperature and duration of contraction and relaxation have also been noted in skeletal muscle following denervation (2, 3). However, such observations have suffered from a lack of quantitative data and have led to inconsistent conclusions. The purpose of the present study was to investigate the nature, extent and causal relationships of the changes in blood flow, temperature and duration of periods of contraction and relaxation in skeletal muscle at various intervals after denervation.

Methods. All experiments were carried out on adult dogs. Denervation was accomplished under ether anesthesia by sectioning the sciatic nerve at the level of the trochanter and by cutting the femoral nerve just below the ligament of Poupart. The unoperated contralateral limb served as a control. Muscle temperatures were measured at various times after denervation by inserting into the belly of the gastrocnemius muscle an iron-constantan junction thermocouple, soldered into a number 20 hypodermic needle. The temperature was calculated from the e.m.f. registered by a Leeds and Northrup potentiometer.

Measurements were made of temperatures in the denervated gastrocnemii at various times after operation and compared with those in their unoperated contralateral controls. In addition, the efficiency of the temperature regulating mechanism in denervated limbs was compared with that in normal limbs. This was done by recording the temperature difference between normal and denervated muscle while the animals were in an environment of 25°C. and −2°C.

In order to compare the effect of application of heat to normal and denervated muscle short-wave diathermy (15-25 megacycles) was applied to both the control and denervated gastrocnemii of 29 animals. Care was exercised to insure uniform technique; the manner of application, dosage intensity and duration of application were the same for both normal and denervated limbs. One group of 11 dogs was treated for 15 minutes with a machine dosage of 1500 m.a. and another

[1] Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

group of 18 animals was treated with a machine dosage of 2000 m.a. The muscle temperatures were taken both before and immediately after diathermy treatments.

The effects of denervation upon the contraction and relaxation times of the gastrocnemius muscle were determined in studies on 8 dogs, each having been subjected to a denervation of one hind limb as described previously. Studies were also made on the muscles both before and after treatments with diathermy. The animals were placed in a supine position with both legs firmly supported at the knees and the toes were attached to recording stylii with the aid of cord and pulleys. Adequate break shocks from an inductorium were delivered to the muscles through needle electrodes, one in the Achilles tendon and the other near the origin of the muscle. The isotonic twitch responses of the experimental and contralateral control muscles were recorded on a rapidly moving kymograph simultaneously with the vibrations of a 100 d.v. electrically-driven tuning fork. Ordinates were constructed on the graphs and the time interval of each phase was read in 0.01 second limits.

Measurements of blood flow were made by means of a bubble-flow meter as described by Dumke and Schmidt (4). Simultaneous bilateral measurements of blood flow were made in most experiments, one in the femoral artery of the denervated limb and the other in the artery of its non-denervated control. The measurements were made with the animals under nembutal anesthesia and with the use of heparin as an anticoagulant. Care was taken to insure the maintenance of blood fluidity by additional injections of heparin. The cannulae were inserted as high as possible in the femoral artery and were matched within narrow limits of internal diameters and interchanged at random to rule out consistent effects of occlusion of the lumen of the artery. In all cases sufficient time was allowed before taking blood flow measurements to obviate ischemic effects which might have resulted from the unavoidable circulatory interruptions during cannulation. For the purpose of studying the immediate effects of denervation on blood flow, the nerves were exposed but not cut until after measurements had been made of the control blood flow in that limb.

Results. The experimental results of the studies on the temperature characteristics of denervated and control muscles are summarized in table 1. These include studies made on 27 animals during the first 3 days following denervation and during a later period of 14 to 50 days after operation. In every case it was found that the temperature of denervated muscle was significantly lower than that of its contralateral control. This was true both for the studies made early and for those made late after denervation. Differences between the temperatures of control and experimental muscles were also present in animals which had been kept at an environmental temperature of −2°C. for 2 hours. In the experiments conducted in the cold environment both denervated and control muscles exhibited a fall in temperature, but the change was more pronounced in the case of the former. It appears that normal muscle, while undergoing a significant drop in temperature in the colder environment, was better able to compensate for a lowered environmental temperature than was the denervated muscle. In

table 1 are summarized the data from studies on 11 dogs relative to the heating effects of diathermy on normal and denervated muscle. These data show that the heating effect is greater in the denervated muscle than in the normal muscle. This was true for two different dosages of diathermy. The lighter dosage, applied for 15 minutes, was insufficient to produce a significant rise in the temperature of control muscle but it caused a significant increase in the temperature of denervated muscle. The larger dosage, applied for 15 minutes, caused significant increases in temperature of both denervated and control muscle, but the increase was greater in the denervated muscle than in the control.

TABLE 1

Average values with standard deviations for the effects of denervation on muscle temperature

EXPERIMENTAL CONDITION	CONTROL	DENERVATED	DIFFERENCE*
	°C.	°C.	°C.
During first 3 days after denervation.....	39.28 ±0.743	38.54 ±0.866	−0.74 ±0.350 t = 6.03
During 14–50 days after denervation.....	38.76 ±0.458	38.07 ±0.707	−0.69 ±0.020 t = 7.95
Animals kept at 25°C..................	39.30 ±0.74	38.5 ±0.99	−0.70 ±0.35 t = 6.04
Animals kept for 2 hours at −2°C.......	38.60 ±0.78	36.60 ±1.47	−1.90 ±1.63 t = 7.57
Before diathermy.......................	38.9 ±0.54	38.2 ±0.66	−0.70
After diathermy at 1500 m.a............	39.1 ±0.47	39.6 ±0.54	+0.50
Before diathermy.......................	38.7 ±0.50	38.1 ±0.74	−0.60
After diathermy at 2000 m.a............	40.2 ±0.70	40.8 ±1.03	+0.60

* Increment of temperature in denervated muscle.

TABLE 2

Effect of denervation on the contraction and relaxation times of the gastrocnemius muscle

EXPERIMENTAL CONDITION	MUSCLE TEMPERATURE	CONTRACTION TIME	RELAXATION TIME	TOTAL TWITCH TIME
	°C.	sec.	sec.	sec.
Unoperated control......	38.6 ±0.47	0.057 ±0.005	0.086 ±0.025	0.143 ±0.004
Denervated..............	38.3 ±0.47	0.073 ±0.015	0.121 ±0.025	0.194 ±0.031
Denervated and diathermy............	40.9 ±0.54	0.057 ±0.0059	0.078 ±0.0156	0.135 ±0.0169

The data concerning the effects of denervation on the contraction and relaxation times of the gastrocnemius muscle of 8 dogs are summarized in table 2. It

was shown that the durations of the periods of contraction and relaxation were significantly prolonged following denervation. Elevation of muscle temperature by means of diathermy treatments was accompanied by a shortening of contraction and relaxation times in denervated muscle. These studies offer evidence contrary to the view that the slower response of denervated muscle is due in a large measure to its lower temperature, since it was found necessary to raise the temperature of denervated muscle by as much as four times its degree of subnormality before the duration of the periods of contraction and relaxation approximated those found in contralateral control muscle.

TABLE 3

Effect of denervation on blood flow. The flow in femoral artery of denervated limb is expressed as per cent of that in contralateral control

NUMBER	DAYS AFTER DENERVATION	DENERVATED / CONTROL	NUMBER	DAYS AFTER DENERVATION	DENERVATED / CONTROL
		per cent			*per cent*
1	0	192	1	27	72
2	0	241	2	27	60
3	0	144	3	28	69
4	0	180	4	29	82
5	0	189	5	31	96
		—	6	45	74
Mean.......		189	7	50	59
			8	52	92
1	3	151			—
2	3	93	Mean.......		76
3	5	141			
4	5	91			
5	7	64			
6	8	120			
7	9	96			
8	9	105			
9	12	102			
10	18	97			
11	19	131			
12	21	74			
		—			
Mean.......		105			

The data from bilateral blood flow studies at various times after unilateral denervation are summarized in table 3. The studies were made on different animals at three periods of time after denervation. The observations designated as "immediate" were made at various times within the first 2 hours after section of both the sciatic and femoral nerves and show that the average blood flow in the femoral artery was almost doubled following denervation. In 12 experiments made in the period of 3 to 21 days subsequent to denervation, 6 showed a greater rate of flow in the control limb and 6 showed a greater flow rate in the denervated limb. In the group studied during the period of 27 to 52 days after denervation, all cases showed a lesser flow in the denervated than in the control limb.

DISCUSSION. The results of these studies were consistent in showing that denervation was followed by changes in the temperature, in the blood flow and in the contraction and relaxation times of the muscles of the limb. In general, denervation of a limb resulted in a subnormal muscle temperature, which was apparent soon after denervation and which persisted for the full period of observation. An increased blood flow rate was noted immediately after denervation, but, as the period of time subsequent to denervation was extended, the flow rate tended to return to normal limits. Further extensions of the period of denervation were accompanied by greatly reduced blood flow rates in the denervated limbs, the decreased blood flow being paralleled by a decreased muscle weight. There were no indications that the blood supply in the denervated limbs was insufficient for the general metabolic needs of the muscle under conditions of the demands put upon it.

The mechanism of the subnormal temperature in the denervated limb is obscure. In these experiments the areas tested had been deprived of both somatic and autonomic innervation. The loss of heat production resulting from absence of tone and of contraction must be considered as a factor along with changes in the circulatory pattern regulating the distribution, dissipation and conservation of heat. This view is supported by the fact that animals placed in a cold environment showed a greater decrease in temperature in the denervated limb than in the normal limb. Moreover, essentially the same difference in temperature was found between denervated and normal muscle during periods in which blood flow measurements showed widely unrelated blood flow rates. These findings suggest that a lowered metabolic rate and heat production, unrelated to blood supply, may be an important factor contributing to the subnormal temperature in denervated muscle. In this connection it is interesting to point out that standard doses of diathermy, applied separately to denervated and normal limbs, caused a consistently greater increase in temperature in the denervated muscles than in the control muscles. This unequal heating effect of diathermy was also noted in periods wherein blood flow measurements showed widely divergent values.

The mechanical response of denervated muscle to single induction shocks showed that the response time was extended in denervated muscle. The relaxation time was prolonged relatively more than the contraction time, but this prolongation did not compare in magnitude with that observed by others (2) in denervated muscle wherein the muscle was activated by longer periods of stimulii and the responses resembled a form of tetanus from repetitive discharges. Doupe (3) concluded that the sluggish response of denervated muscle was due in a large measure to its subnormal temperature. However, in the present studies, temperature differences would account for only a small part of the prolongation of response, since it was necessary to elevate the temperature of denervated muscle by approximately four times its degree of subnormality before response times were within the range of those for control muscle. These observations lead to the conclusion that the over-all mechanical response of denervated muscle is inherently slower than that of normal muscle even when both the stimuli pattern and the temperature are the same.

It is well to point out the limitations of the technique used to measure blood flow rates before attempting any analysis of the effects of denervation upon the circulatory pattern. The bubble-flow meter permitted observations to be made only upon the flow rate in the femoral artery, alone, and did not give any information concerning blood flow in vessels supplied by other arteries in the limb. It was not possible to deduce the state of blood flow in bone, connective tissue, muscle and other specialized regions supplied by the artery. Moreover, it is recognized that the procedures required general anesthesia, the use of heparin as an anti-coagulant and the sectioning and cannulation of the artery itself. These conditions in themselves certainly affect blood flow rates in the vessels concerned; it could only be assumed that such factors affected denervated and control limbs to the same degree and in the same direction.

The increase in blood flow rate which occurred immediately upon the sectioning of the nerve supply of the limbs can be ascribed to loss of vasomotor control. The gradual return of blood flow rates to within normal control limits was completed too early to be ascribed to re-innervation of the limb. The decrease in blood flow rates to subnormal levels in more extended periods of denervation could not be ascribed to lowered temperatures in the muscles for two reasons: first, that blood flow rates in the denervated limbs could not be returned to normal by raising the muscle temperature to normal and above-normal levels by means of diathermy and other thermogenic agents; second, that such subnormal temperatures in denervated muscle were established early in the post-denervated periods, during which all observations indicated an elevated blood flow rate.

The time of onset of the reduced blood flow is in accord with the effects postulated by the law of denervation, wherein an increased peripheral resistance may result from the response of sensitized structures in the denervated vessels to epinephrin and other vasoconstricting agents. Another factor which must be considered is that of a decrease in vasodilatation stimulii mechanisms associated with a lowered metabolic level in the atrophic muscles. It is of some significance that the decreased blood flow noted in the extended periods of denervation were commensurate with the degree of muscle atrophy existing in each animal at the time of blood flow measurement. The magnitude of the reduction in blood flow was not such as to suggest that ischemic states exist in the tissues of the denervated limb.

In view of the paucity of information concerning the factors regulating the production of heat in tissues by means of diathermy, it is hazardous to venture an opinion as to why diathermy consistently produced a greater temperature rise in denervated muscle than in normal muscle. It has been pointed out that such unequal heating effects cannot logically be explained totally in terms of a decreased dissipation of heat by reduced blood flow, because it was found impossible to correlate the blood flow pattern at various post-denervated periods with inequalities of heating in denervated and normal limbs. It appears more probable that the altered chemical and physical states in denervated muscles caused them to react differently to the thermogenic properties of diathermy.

SUMMARY

1. Studies were carried out on dogs concerning the effects of denervation upon blood flow, temperature characteristics and contraction and relaxation times of the gastrocnemius muscle. The observations were made at various times after denervation and the unoperated contralateral limb served as a control.

2. Denervation was followed by an immediate increase in blood flow through the femoral artery, the flow returning to normal after several days of denervation. More extended periods of denervation resulted in subnormal blood flow values which appeared to parallel the extent of muscle atrophy.

3. Denervated muscles exhibited a subnormal temperature and responded to diathermy with temperature increases greater than those observed in normal muscle.

4. The twitch response of denervated muscle to induction shocks was slower than that of control muscle. This was due only in part to its subnormal temperature.

The subnormal temperature, increased susceptibility to diathermy and the sluggish response of muscle at various times after denervation did not appear to be related to changes in blood flow.

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AN ANALYSIS OF THE CAROTID SINUS CARDIOVASCULAR REFLEX MECHANISM[1, 2]

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The present concept of the carotid sinus mechanism has changed but little since the early work of Hering (1) and Heymans (2, 3). It is generally agreed that the vagus plays the major rôle in cardiac restraint arising from the carotid sinus pressoreceptors and that the sympathetic accelerators are involved to a lesser but definite degree. On the other hand, the depressor response to carotid sinus stimulation is largely effected through the activity of the sympathetic system.

The elusive question which remains, however, is how the vagi and sympathetics interact in time sequence in reflex control of the cardiac rate by the carotid sinus. Indeed, the exact mechanism by which the vagus is controlled in the reflex response of the heart is not clear.

Despite the fact that in vagotomized animals the vasomotor effect is still elicitable, the manner and extent of vagal involvement in this response is vague. It was with the clarification of these problems in mind that the present work was undertaken.

METHODS. Forty experiments were carried out on healthy mongrel dogs ranging in weight from 5 to 13 kgm. They were anesthetized with nembutal (35 mgm. per kgm.) given intraperitoneally. The carotid sinus was stimulated by a sudden increase of pressure within the sinus, maintained for a period of 45 seconds.

The common carotid arteries were exposed at the level of the cricoid cartilage. The internal carotid, occipital, external carotid, and lingual branches, and any small aberrant arteries were ligated (4). Through a cannula inserted into the common carotid artery, a concentrated solution of hemostatic globulin[3] was injected into the isolated sinus to produce embolization of the fine vessels. After a few minutes, the large clot in the cannula was washed out, and the cannula was connected to a pressure bottle filled with physiological saline.

A sudden increase of the intrasinual pressure was obtained by unclamping the connecting tube from the pressure bottle, which could be previously pumped to any given level up to 250 mm. Hg. These changes in the intrasinual pressure were recorded by a critically damped bellows manometer in parallel circuit with the sinus pressure tubing.

The cardiovascular response to carotid sinus distention was recorded from the femoral artery with a mercury manometer. The heart rate as registered on the kymograph was verified frequently by counting of the heart beat.

[1] This work was aided by a grant from the Josiah Macy, Jr. Foundation.

[2] A preliminary report of this work appeared in Fed. Proc. 6: 222, 1947.

[3] Hemostatic globulin was kindly supplied by the Lederle Laboratories, Inc.

Experiments performed on the completely sympathectomized dogs were carried out approximately 6 to 8 weeks after surgical removal of the sympathetic chains (5). The procedure used for excising the aortic depressor nerves was that described by Koch (6). The nerve is indentified as a small branch of the vagosympathetic trunk, which can be separated for about 1 to 2 cm. before it joins with the superior laryngeal nerve in the region of the carotid bifurcation. On several occasions in our experience, such a branch was not indentifiable. Under such circumstances, the motor part of the vagus was identified by electrical stimulation and all other fibers were cut. The latter procedure was termed "vagal deafferentation".

Results. To obtain a marked vagal bradycardia, it is essential to raise the intrasinual pressure very suddenly. By increasing the pressure in a step-wise manner, the vagal bradycardia is not clearly evident. This is interesting, especially since the extent of the depressor response is not appreciably altered with different rates of pressure change. The optimal intrasinual pressure rise for a maximal response in the heart and vascular system is 200 to 220 mm. Hg; no further reduction in heart rate or blood pressure is obtained when the pressure is increased beyond this range. In all experiments performed in the present series, intrasinual pressures of the order of 240 mm. Hg were used to insure maximal stimulation.

1. *Vagal and sympathetic bradycardia.* Like all physiological phenomena, the carotid sinus cardiovascular response is of variable degree from animal to animal. The 4 characteristic types of response, presented in figure 1, were selected according to the duration and magnitude of the vagal component observed during the 45 seconds of sustained sinus distention. In 19 experiments in which both sinuses were stimulated, approximately 10 per cent showed type I response, 35 per cent type II, 15 per cent type III, and 40 per cent type IV.

With the elimination of the vagus, slowing of the heart on sinus distention is greatly reduced (table 1). The onset of bradycardia is no longer immediately apparent. However, the sympathetic component plays an increasingly greater rôle and accounts for most, if not all, of the cardiac slowing in the third 15 second period of sinus stimulation. The maximum sympathetic slowing, about 20 per cent of the control heart rate, compares favorably with the results of Winder (7), with the exception that in the present series this value remains unchanged during artificial respiration.

2. *Vagal bradycardia in functionally sympathectomized animals.* The question as to whether the marked vagal slowing following sinus distention is dependent upon the concomitant change in the sympathetic activity has not been satisfactorily answered. Our experiments were performed on three types of animals with the sympathetic nervous functions excluded: *a*, chronic sympathectomy (fig. 2); *b*, acute low cervical cord transection (fig. 3), and *c*, under the influence of a sympathicolytic drug, dihydroergotamine (D. H. E. 45)[4] (fig. 4). The procedures employed in excluding the sympathetic function by cord transection and by injection of a sympathicolytic drug, though they involve more than simple elimina-

[4] Dihydroergotamine, D. H. E. 45, was kindly supplied by Sandoz Chemical Works, Inc.

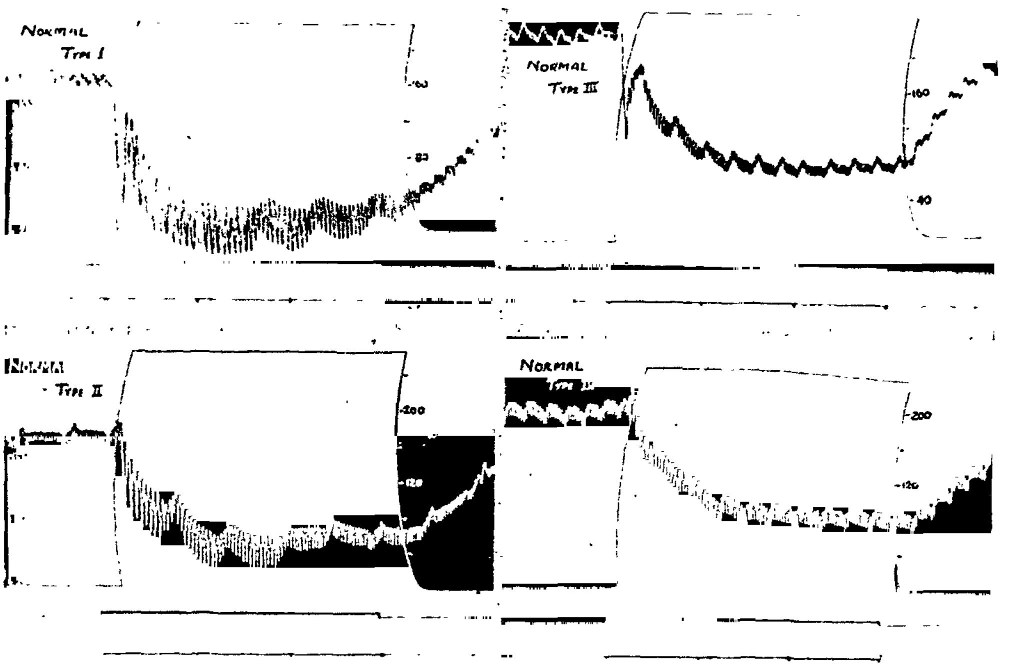

Fig. 1. Four characteristic normal cardiovascular responses to simultaneous bilateral carotid sinus distention. Blood pressure level is indicated on the left. Intrasinual pressures are marked on the descending limb in each record. Time in 15 seconds. Type I—very marked, prolonged bradycardia; type II—transient marked bradycardia; type III—momentary vagal block followed by moderate bradycardia; type IV—moderate bradycardia.

TABLE 1

Comparison of the cardiac slowing following carotid sinus distention in normal and vagotomized dogs

	FIRST 15 SECONDS		THIRD 15 SECONDS	
Combined (type I)	−67%		−43%	
Sympathetic (after vagotomy)	−9%		−23%	
Vagal		−58%		−20%
Combined (type II)	−48%		−20%	
Sympathetic (after vagotomy)	−7%		−19%	
Vagal		−41%		−1%
Combined (type III)	−42%		−12%	
Sympathetic (after vagotomy)	−13%		−13%	
Vagal		−29%		1%
Combined (type IV)	−21%		−11%	
Sympathetic (after vagotomy)	−5%		−13%	
Vagal		−16%		2%

tion of the sympathetic nervous structures, have the advantage that control responses can be obtained immediately before these procedures are carried out. The

results of these experiments, in which cardiac slowing is only under vagal control[5], are summarized in table 2. From these data, it can be seen that sympathetic exclusion does not interfere with the initiation of the sino-vagal response. On elimi-

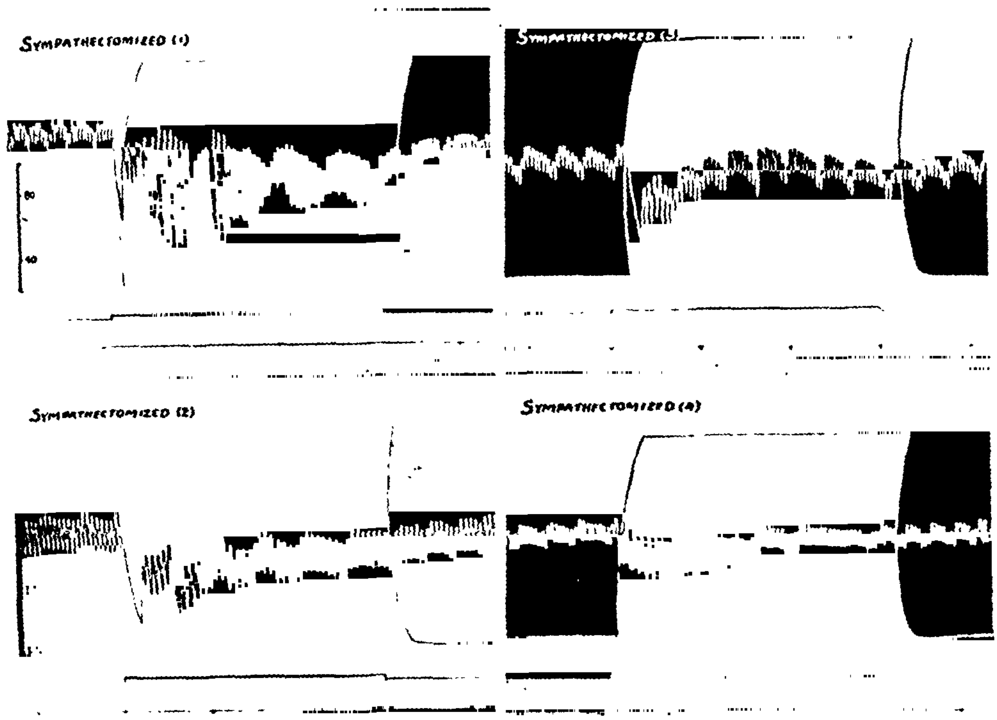

Fig. 2. Four varieties of vagal bradycardia on bilateral sinus distention in chronic sympathectomized dogs. Compare with the vagal component of the responses in figure 1.

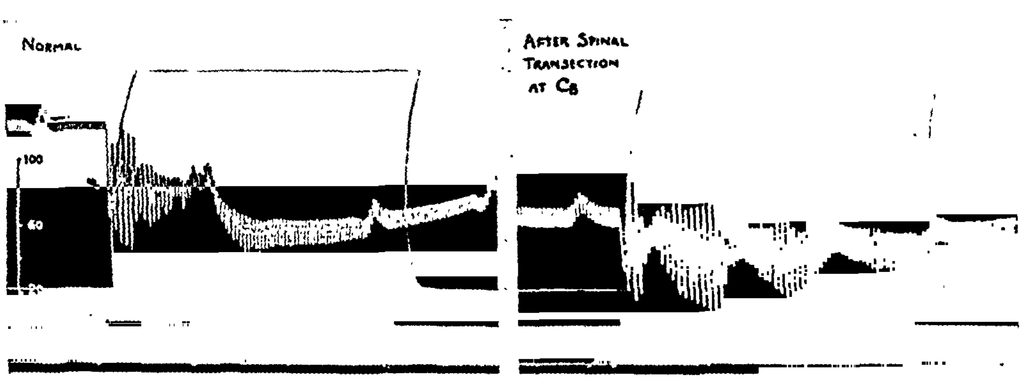

Fig. 3. Comparison of the responses to bilateral sinus distention before and after spinal transection at C_8. The sino-vagal bradycardia after cord section is larger and more prolonged than before.

nation of the late sympathetic slowing of the heart, the vagal effect persisted in most cases during the third 15 second period of the sustained sinus distention (table 2)

[5] The cardiac slowing in functionally sympathectomized animals is purely vagal, since following vagotomy stimulation of the sinus produces no change of heart rate despite inhibition of the respiration (fig. 5).

3. *Vagal deafferentation and the sino-vagal bradycardia.* It has been shown that vagal bradycardia disappears rapidly in spite of continued sinus distention (fig. 1 and table 1). After surgical exclusion of the vagal afferents sinus stimulation produces a marked effect not only in potentiating but also in prolonging the vagal cardiac slowing (fig. 4). In a dog in which the aortic depressor nerves were excluded three weeks previously, the heart showed an escape phenomenon strikingly

TABLE 2

Vagal cardiac slowing following carotid sinus distention in functionally sympathectomized dogs

		FIRST 15 SECONDS	THIRD 15 SECONDS
In sympathectomized dogs	(1)	−40%	−24%
	(2)	−35%	−14%
	(3)	−23%	0%
	(4)	−27%	−4%
In high spinal dogs	(1)	−41%	−15%
	(2)	−36%	−18%
In dogs with D.H.E. 45	(1)	−38%	−16%
	(2)	−25%	−13%
	(3)	−24%	−16%

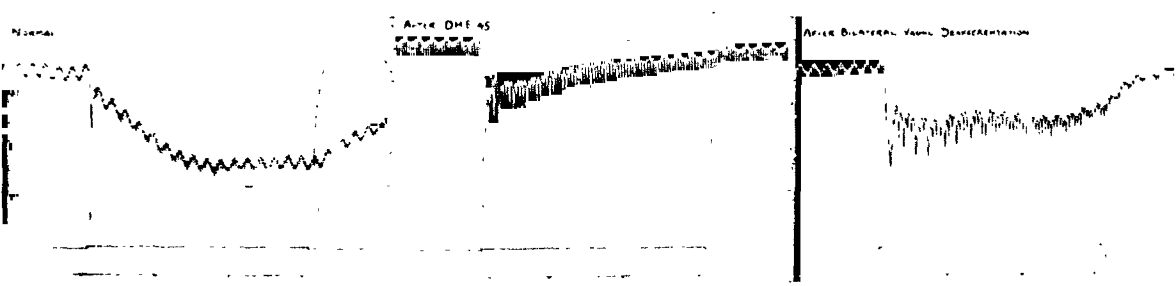

Fig. 4. Effect of D. H. E. 45 (dihydroergotamine) and vagal deafferentation on the sino-vagal bradycardia. Left: control cardiovascular response to bilateral sinus distention. Center: after intravenous injection of D. H. E. 45 (0.5 mgm. per kgm.). Note the intensified and prolonged bradycardia. Right: after bilateral vagal deafferentation. The bradycardia is further intensified.

similar to that occurring after stimulation of the peripheral vagal trunk (fig. 6). This serves to emphasize the fact that despite elimination of the important correcting influence of the aortic depressor nerve on the heart rate, some vagal adaptation still occurs.

4. *Bilateral vagotomy and the sinus depressor response.* The depressor response to sinus distention is sometimes enhanced and at other times lessened when the vagal trunk is cut bilaterally. However, if the motor component of the vagus is alone excluded, either by surgery or by atropinization (fig. 7), not only is a smaller depressor response obtained but its onset is also more gradual. On additional

exclusion of the vagal afferents, the depressor response to sinus distention becomes definitely intensified (fig. 7).

Discussion. In experiments on carotid sinus reflexes chloralose has come to be the preferred anesthesia, because both pressure and chemical reflexes appear to be more active with this than with other narcotics (3). However, Wang and Nims (8) have recently found that in cats the respiratory response to CO_2 is depressed as greatly by chloralose as by nembutal. It is our experience in the study of the carotid sinus cardiovascular reflex that nembutal is a satisfactory anesthetic. On

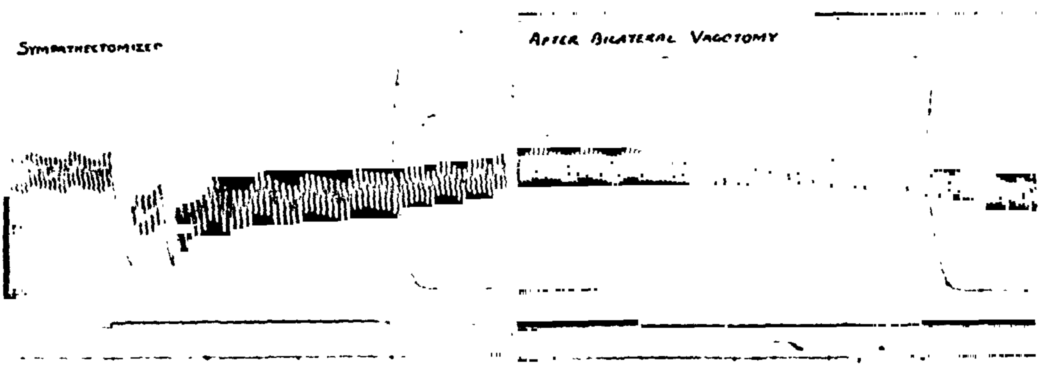

Fig. 5. Complete absence of cardiovascular response on bilateral sinus distention after vagotomy in a sympathectomized dog. Note the inhibition of respiratory variations in record on right.

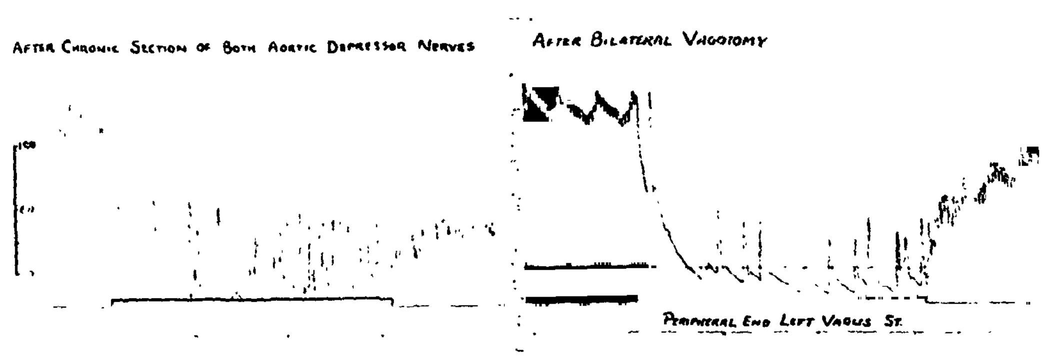

Fig. 6. Comparison of vagal adaptation on bilateral sinus distention with "vagal escape" (record on right), in an animal with chronic aortic depressor nerve section.

bilateral sinus distention in a normal nembutalized dog, we have obtained a maximum reduction of heart rate as much as 67 per cent of the control rate and on several occasions a reduction of blood pressure as much as 150 mm. Hg. Nembutal has, besides, the further advantage of giving a steady anesthesia which is essential in obtaining consistent responses to repeated stimulation of the sinus with with identical pressures.

Many workers have chosen to perfuse the sinus with oxygenated blood. However, we have found that the sinus pressoreceptors are very resistant to anoxia, since with physiological saline as the perfusion fluid, our carotid sinus preparations have given marked and reproducible responses for a period of at least 3 hours. It is therefore considered unnecessary to use a more complicated technic.

The importance of an adequate stimulus as regards both its temporal and spatial values should be stressed. The sharp vagal response seen immediately following distention is rare, except when the stimulus is of an intense and sudden nature, and applied simultaneously to both sinuses. It is this type of stimulus that has enabled us to analyze the carotid sinus reflex for both vagal and sympathetic components.

Time relationships of vagal and sympathetic bradycardia. Hering (1) observed that the cardiac reflex is of more sudden onset than the vasomotor reflex and that it is much more poorly maintained. Our experiments confirm this observation. It can be further stated of the heart reflex (table 1) that *a*, the combined effect of vagal and sympathetic components is always greatest during the early moments of the stimulation period; *b*, the vagus accounts for most of the slowing immediately following stimulation; and *c*, the onset of decreased sympathetic activity is slow but accounts for most of the cardiac slowing as stimulation persists. Our findings substantiate further the evidence for the reciprocal activity of the vagus and sympathetic cardiac accelerators (9) in response to changing sinus pressure, sympathetic function becoming dominant as the vagal influence wanes.

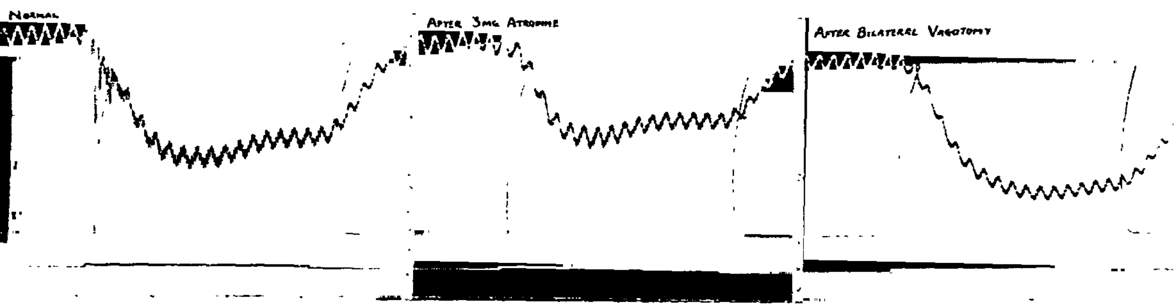

Fig. 7. Effect of successive exclusion of the motor and afferent vagal influences on the depressor response to bilateral sinus distention. Artificial respiration. Left: control response to bilateral sinus distention. Center: after intravenous injection of 3.0 mgm. of atropine to exclude the parasympathetic efferents. Note the slower onset and diminished depressor response. Right: after bilateral vagotomy. Additional elimination of the vagal afferents (aortic depressor nerve) enhances the sinus depressor response and causes the vasomotor adaptation to disappear.

Dependence of vagal slowing on the sinus sympathetic depressor response. To what extent is vagal slowing dependent upon its association with the sympathetic depressor effect to which it is so closely bound in the usual response to stimulation of the carotid sinus pressoreceptors? This question has not yet been answered satisfactorily since there is no clear cut experiment described in the literature which completely separates the reflex vagal and sympathetic connections. That the blood flow through the brain is not involved has been shown by Heymans (3) who demonstrated by crossed circulation experiments that variation of the cerebral blood flow is not a factor in producing the reflex vagal effect. We found that the vagal cardiac response immediately following bilateral sinus distention, in all types of functionally sympathectomized animals used, was as marked as that elicited in the intact animal. It is clear then that the initiation

of the sino-vagal bradycardia is quite independent of changes in the sympathetic activity. Furthermore, our data indicate that in functionally sympathectomized animals vagal slowing is much prolonged (table 2). This is in contrast to the more transient nature of vagal bradycardia obtained on sustained sinus stimulation in the majority of the experiments on dogs with intact sympathetics (table 1). This observation is interesting in view of the following discussion on the importance of the aortic depressor impulses in the sinus vagal adaptation.

Mechanism of adaptation of the sino-vagal bradycardia. The poorly maintained sino-vagal bradycardia has been previously reported by Hering (1) and also by Schneyer (10). Their observations were confirmed in the experiments in which we found that vagal bradycardia rarely persisted beyond the first 15 seconds of sustained sinus distention. The rapid disappearance of the vagal cardiac reflex is generally termed adaptation. It may be due either to one, or to a combination of two or more of the 4 mechanisms: *a*, a property of the sinus pressoreceptors; *b*, the aortic depressor nerve buffer mechanism; *c*, some process of adaptation of the centers in the brain stem; and *d*, the nature of the effector organ, the heart.

Bronk and Stella (11) observed that the pressoreceptors in the carotid sinus are of the type that shows little adaptation. Further, if it were assumed that the transient nature of the vagal response could be accounted for by the small amount of adaptation in the pressoreceptors alone, it would follow that the sympathetic inhibitory effect on the blood pressure should also exhibit an equally marked adaptation, which ordinarily is not seen. Indeed, in contrast to the difficulty in obtaining a clear cut vagal bradycardia, the sympathetic depressor response is of the same magnitude whether the pressure within the sinus is raised suddenly or slowly. However, it is possible that the slight adaptation of the receptor organs may play a rôle in the rapid disappearance of the vagal bradycardia.

The idea that the aortic arch buffer mechanism is responsible for vagal adaptation has received wide attention (6, 10). In animals with sympathetics functionally excluded, there appears to be a prolonged vagal effect following sinus stimulation (table 2). This finding may be explained by the fact that following functional sympathectomy the sympathetic depressor response to sinus stimulation is absent (see fig. 2), thereby eliminating in large part the stimulus to the receptors of the aortic depressor nerve. This sino-vagal bradycardia is further intensified if in addition the vagal afferents are sectioned. But the fact remains that in all instances after deafferentation of the vagus, sinus vagal slowing still shows a considerable degree of adaptation. This suggests that the process of adaptation may in part be central. In support of this, Bronk et al. (12) observed a return of sympathetic discharge during continued stimulation of sinus pressoreceptors. They believe that this is partly a central phenomenon, since vagotomy (exclusion of depressor fibers) does not prevent the return of sympathetic discharge, but only prolongs the inhibition. However, as far as the sinus vagal reflex is concerned, it is important to note that the heart itself may escape the vagal restraining influence and show normal or ectopic rhythms. Figure 6

shows a close resemblance between the bradycardia resulting from direct vagal stimulation and that obtained by distention of the sinuses in a dog in which the aortic depressor nerves were excluded previously. Further substantiation of the fact that the effector organ may play a rôle in adaptation of the vagal reflex is made by several experiments in which the type III response (fig. 1) was obtained; exclusion of the vagal afferents did not prolong the bradycardia on sinus stimulation. In one of these animals, stimulation of the peripheral vagus gave no strong vagal block, thus indicating that the failure in maintaining the sinus cardiac reflex may be related to the familiar yet unexplained phenomenon of "vagal escape".

Effect of vagotomy on the sinus depressor response. It has long been known that the depressor response in the carotid sinus reflex is effected through an inhibition of the sympathetic system. The effect of vagal impulses upon this depressor response is not clear. Indeed, the influence of bilateral vagotomy on the carotid sinus depressor response is a varied one, apparently due to the fact that in vagotomy both the motor and afferent components are severed. Exclusion of the parasympathetic vagal component alone results, on sinus stimulation, in a depressor response that is of gradual onset and not as marked as in the normal. The difference can be explained by the absence of marked cardiac slowing which contributes also to the fall of the blood pressure. On the other hand, the effect of vagal deafferentation is to intensify the sinus depressor reflex. In addition, it decreases but does not eliminate, in most cases, the small adaptation of the depressor response which occurs after 30 seconds of continued sinus stimulation. Thus, the end result after complete vagotomy depends on the balance which exists between these two vagal components, the afferent and efferent.

SUMMARY AND CONCLUSIONS

Experiments were carried out on dogs anesthetized with nembutal, in which the carotid sinus was vascularly isolated and stimulated with a sudden increase of pressure.

Vagal activity as shown by cardiac slowing is most marked immediately following sinus distention; the bradycardia due to sympathetic inhibition, on the other hand, appears slowly and becomes the major restraining influence as the vagal effect diminishes. Our experiments demonstrate clearly that the vagal and sympathetic tonic activities respond to changing sinus pressure in a reciprocal manner but that the dominance of each of these influences on the effector makes its appearance in a different phase of time sequence. We have demonstrated also that the vagal cardiac slowing is a discrete response and that it is not dependent on the sympathetic depression which normally invariably occurs in the sinus cardiovascular reflex.

Evidence has been presented to show that the rapid disappearance of the sino-vagal bradycardia is due not only to the buffering effect of the aortic depressor nerves but also to a property of the effector organ, the phenomenon of "vagal escape".

It has been shown that there exists a balance, in the sinus depressor reflex, be-

tween the afferent and efferent vagal influences; elimination of the former enhances the response, whereas exclusion of the latter lessens it.

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DECUSSATION OF THE PATHWAYS IN THE CAROTID SINUS CARDIOVASCULAR REFLEX: AN EXAMPLE OF THE PRINCIPLE OF CONVERGENCE[1]

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In the past few years, investigators of the carotid sinus cardiovascular mechanism have produced much evidence in favor of the synergistic relationship between the sympathetic and parasympathetic nervous systems (1). A study of the respective rôles and the relative importance of these systems with reference to the carotid sinus reflexes has been reported recently from this laboratory (2). One aspect of the problem which is in need of clarification is the question of decussation of the carotid sinus reflex pathways. Although the crossing of the vagal component has been the object of study by many workers, the results are still controversial. On the other hand, decussation of the pathways of the sinus vasomotor reflex has been clearly demonstrated, but the level of decussation has not been indicated. The present study was undertaken to investigate the decussation of these central pathways and their relation to the principle of convergence.

Methods. In dogs anesthetized with nembutal the carotid sinus was exposed and stimulated according to the technic described in a previous paper (2). In brief, the sinus was isolated as a cul-de-sac and perfused with physiological saline. The stimulus was a sudden distention accomplished by raising the perperfusion pressure within the sinus from 0 to approximately 240 mm. Hg; this was recorded by a critically damped bellows manometer. The reflex cardiovascular response was recorded from the femoral artery with a mercury manometer.

For sympathectomies, the technic of Cannon et al. (3) was used.

Results. Distention of the right carotid sinus usually produced more slowing of the heart and a greater fall in blood pressure than an equivalent distention of the left sinus (tables 1 and 2). In the first 15 seconds of simultaneous bilateral sinus stimulation the bradycardia was invariably greater than the sum of the reductions in heart rate obtained on separate stimulation of right and left sinuses (table 1). On the other hand, the maximal vasomotor response to simultaneous distention of both sinuses was, as a rule, less than the sum of the responses obtained from stimulation of both sinuses separately (table 2). Indeed, in several experiments stimulation of both sinuses produced only a slightly greater depressor response than that of the right or the left sinus alone. It is true, however, that when both sinuses were stimulated at the same time, the depressor response was usually more sustained.

The question of crossing in the vagal cardiac reflex pathways has been studied upon chronically sympathectomized dogs, for in these animals the complication

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of sympathetic involvement is eliminated. The results of a typical experiment are indicated in figure 1. The records show that after right vagotomy, stimulation of the right sinus produced a slight bradycardia; this showing disappeared following left vagotomy even though both sinuses were simultaneously distended. In the same animal after right vagotomy, a much greater slowing of the heart occurred when both sinuses were stimulated simultaneously than when they were stimulated separately (fig. 2).

That the sinus vasomotor reflex pathways cross was demonstrated on dogs in which the sympathetic chains on one side had been previously removed. While the vasomotor depression was not very marked in these preparations, an equally

TABLE 1

Reduction of the heart rate during the first 15 second period of sustained distention of the right sinus, the left sinus, and both sinuses

	CHANGE OF HEART RATE IN BEATS PER 15 SECONDS		
	Right sinus	Left sinus	Both sinuses
(1)	−15	−9	−26
(2)	−15	−4	−25
(3)	−17	−10	−29
(4)	−13	−2	−19
(5)	−10	−6	−17

TABLE 2

Maximal fall of the mean blood pressure in response to sustained distention of the right sinus, the left sinus, and both sinuses

	MAXIMAL DROP IN BLOOD PRESSURE IN MM. Hg		
	Right sinus	Left sinus	Both sinuses
(1)	−80	−70	−130
(2)	−95	−90	−120
(3)	−80	−60	−110
(4)	−80	−80	−120
(5)	−75	−55	−90

good response was obtained from either sinus (fig. 3). Decerebration did not alter the reflex vasomotor response in any appreciable manner (fig. 4). After acute hemisection of the spinal cord at C_2, distention of the sinus on the side of the cord lesion was much less effective in producing a fall in blood pressure than was distention of the sinus of the other side. The results of such an experiment are illustrated in figure 3, in which it is seen in a right hemisympathectomized dog that whereas both sinuses were equally effective in producing a depressor response before cord section, only a very small response was elicited from the right sinus after right cord hemisection at the level of C_2.

Discussion. One controversial question concerned with the carotid sinus

cardiac reflex is whether the vagal component of the bradycardia following sinus distention is mediated through one vagus or through both vagi. Hering (4) was of the opinion that the sinus vagal reflex is largely ipsilateral, whereas Izquierdo (5) claimed that the vagal efferents on both sides are equally activated on stimulation of either of the carotid sinuses. On the other hand, Heymans et al. (1) maintained that the sinus vagal response is obtained strictly through the vagus on the same side. One source of confusion, as Heymans has previously pointed out, is that it is difficult in the intact animal to exclude what may appear to be a contralateral vagal effect since slight slowing of the heart is also effected through sympathetic inhibition. Tournade and Malméjac (6) have attempted

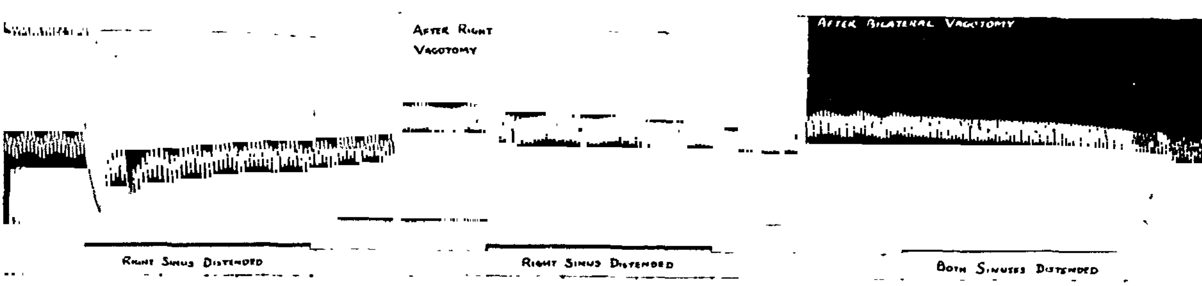

Fig. 1. Crossed sino-vagal bradycardia in a chronically sympathectomized dog. Left: right sinus distended, vagi intact. Center: right sinus distended after right vagotomy. Note the slight bradycardia. Right: both sinuses distended after both vagi sectioned. Note the lack of bradycardia despite continued inhibition of respiratory variations during stimulation.

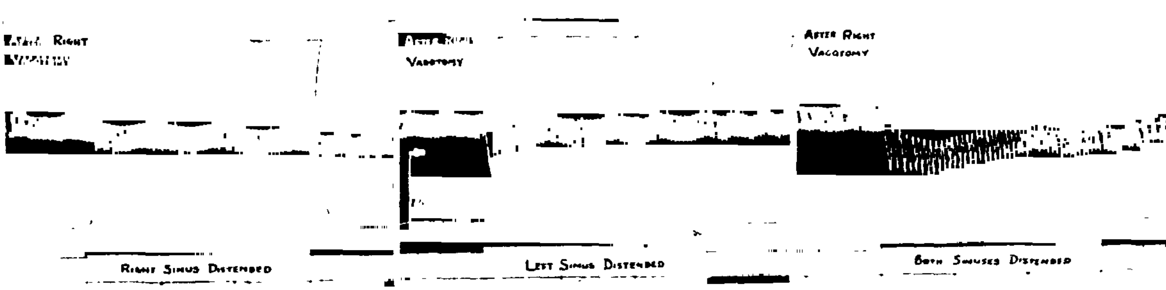

Fig. 2. Comparison of the vagal bradycardia obtained on separate distension of the right and left sinuses, and that obtained on simultaneous distention of both sinuses in a sympathectomized, unilaterally vagotomized dog.

to eliminate this complication by using animals in which the stellate ganglia had been removed. Such preparations, however, are not adequate, since the heart is known to receive sympathetic fibers from other upper thoracic ganglia; and further, the heart may be influenced secondarily by the effects of sympathetic inhibition on other parts of the vascular system. In our completely sympathectomized dogs in which one vagus was sectioned, distention of the carotid sinus on the vagotomized side still produced a small, but definite, bradycardia. This bradycardia can not be accounted for by other effects of sinus stimulation, since after exclusion of the opposite vagus, there was no longer any change of heart rate despite continued inhibition of respiration (fig. 1). If no decussation of

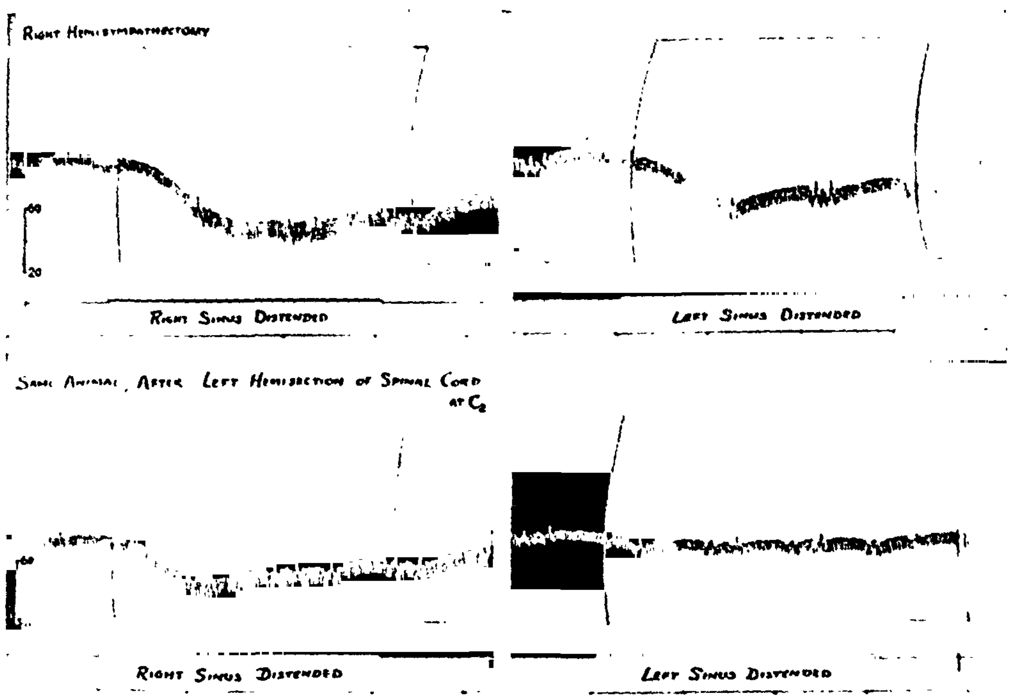

Fig. 3. Effect of hemisection of the spinal cord at C_2 on the sinus depressor response in a hemisympathectomized dog. Atropine, 3 mgm. I. V. Upper: control depressor responses on right and left sinus distention. Lower: responses obtained after hemisection of the spinal cord on the left side. Note the markedly decreased response on distention of the left sinus

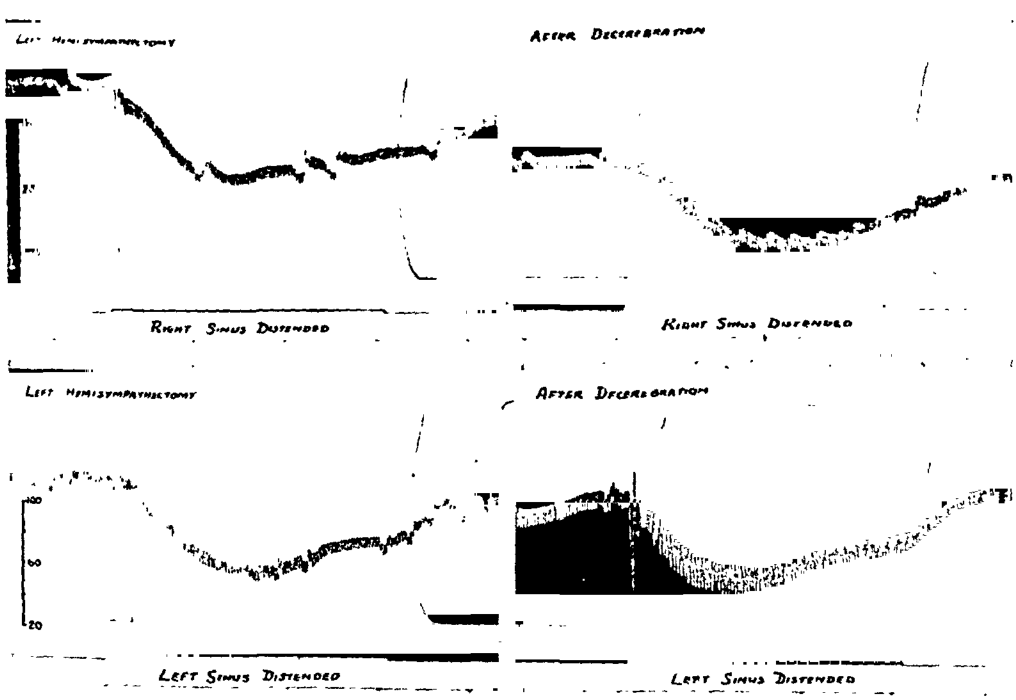

Fig. 4. Effect of midbrain decerebration on the sinus depressor response in two dogs with left hemisympathectomy. Note that the sinus depressor response remains largely unaffected.

pathways occurred, the sympathectomized, unilaterally vagotomized dog would show the same cardiac slowing whether the sinus opposite to the side of vagotomy were distended alone, or both sinuses distended simultaneously. The fact that there is a greater slowing when both sinuses are stimulated together indicates clearly that there is a definite crossed vagal component. However, it should be pointed out that it is not possible to determine from this type of experiment whether the crossing occurs in the afferent or in the efferent limb of the reflex arc.

In contrast to the controversial opinion as regards the sinus vagal reflex pathways, there has not been any question as to the bilateral nature of the sympathetic response to distention of a single sinus. By vacuum tube recording of impulses in one of the small sympathetic accelerator nerves in the cat, Bronk et al. (7) have demonstrated that distention of either sinus produces inhibition of the sympathetic impulses to the heart. The fact that in our experiments distention of a single sinus produces almost as marked a fall in blood pressure as distention of both sinuses suggests an extensive synaptic overlapping of the central pathways of the sinus depressor reflex arc. This is proved with certainty by experiments on hemisympathectomized dogs in which stimulation of either sinus produces a depressor response. The problem that remains is to determine the level of decussation of the sinus vasomotor pathways within the central nervous system.

It has long been known that the carotid sinus reflex is mediated at the myelencephalic level. However, there is no evidence to indicate that in the intact animal, the diencephalon does not influence the sinus depressor response. For our purpose of studying decussation, therefore, it is essential first to exclude the rostral structures as one of the sources of decussation, since there is considerable evidence to show that the descending pathways from the hypothalamus cross at the diencephalic level (8, 9). The evidence that in a hemisympathectomized dog the sinus depressor response is not appreciably modified following midbrain decerebration indicates at least that sympathetic decussation rostral to the medulla oblongata is not essential for the crossed vasomotor response (fig. 4). The fact that in a hemisympathectomized dog, hemisection of the spinal cord at C_2 markedly affects the depressor response of the sinus on the same side as the cord hemisection (fig. 3) suggests further that the major part of the decussation occurs at a level below C_2. This is in conformity with the earlier findings of one of us (9) that sympathetic decussation, as demonstrated by direct stimulation of the myelencephalon, occurs in the spinal cord. The question as to how much decussation occurs in the medulla can not be positively answered, since there yet remains some response to sinus stimulation on the side of cord hemisection.

In the course of our study on the problem of decussation, we have noted a very interesting phenomenon, heretofore not described for the central autonomic nervous system. It is perhaps identical with that which Sherrington (10) has demonstrated in his studies of the reflex activities of the spinal cord. The overlapping synaptic innervation of one neurone by a number of fibers is known as the principle of convergence. The consequent manifestation of this convergence is either facilitation or occlusion. It has been observed in the present work that

the bradycardia following bilateral sinus distention is invariably greater than the sum of the reductions in heart rate obtained from the two sinuses distended separately (table 1). It is difficult to assume, nevertheless, that the phenomenon of facilitation definitely occurs in view of the complication of the crossed sino-vagal response and of the bradycardia resulting from sympathetic inhibition. In several of our sympathectomized, unilaterally vagotomized dogs, reduction of heart rate during the early period of bilateral sinus distention greatly exceeded the simple sum of the reductions of the heart rates obtained by separate stimulaion. This is illustrated in figure 2, in a sympathectomized dog after right vagotomy; the reduction of heart rate in the first 15 seconds of right sinus distention is 2 beats, that of the left sinus is 3 beats, whereas that of both sinuses distended simultaneously is 8 beats. Since in this experiment only one vagus is intact, the process of facilitation probably occurs in the myelencephalon. It appears from the above that while only a small cardiac response can be obtained by stimulation of the sinus on the vagotomized side, the afferent impulses from this sinus set up a considerable "zone of discharge" in a "subliminal fringe" in the contralateral medulla, thereby causing on simultaneous stimulation of the remaining sinus, a greater summated response than can be obtained by simply adding the separate responses from each of the sinuses. It may be assumed that this phenomenon is similar to the facilitation that is seen in the spinal reflexes.

The sinus depressor reflexes obtained in our experiments demonstrate the occlusion phenomenon. The fall in blood pressure which occurs on bilateral sinus distention is very much less than the sum of the individual vasomotor responses (table 2). Except for the fact that vasomotor depression involves an inhibitory process, the experimental information cited closely parallels the classical representation of the occlusion phenomenon in the spinal reflexes. Although Eccles (11) has shown that occlusion may occur also in a peripheral sympathetic ganglion, this convergence phenomenon as demonstrated in the present experiments can only occur in the central nervous system since it is effected through the mechanism of active inhibition.

SUMMARY AND CONCLUSIONS

It has been shown that in the vagal cardiac response to carotid sinus distention there is a small crossed component. This was demonstrated in completely sympathectomized dogs in which the complication of bradycardia due to sympathetic inhibition was eliminated.

The sinus vasomotor response, on the other hand, has a large crossed component. This decussation occurs mainly in the spinal cord.

The principle of convergence has been demonstrated: facilitation in the case of the sinus vagal reflex and occlusion in the case of the sinus vasomotor reflex.

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THE CHANGE IN SPECIFIC GRAVITY OF THE BLOOD PLASMA OF THE RAT DURING SEVERE WATER PRIVATION[1]

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Rose, Stuckey, Mendel, and Cowgill have reported a marked increase in the hemoglobin value of the dog's blood during water privation. They comment, "... the results of the present study fail to support the contention that the organism possesses a great factor of safety with respect to fluid balance when it is deprived of water" (5, p. 138). Flemister has observed only a slight reduction in the plasma volume of the rabbit following dehydration effected by the intraperitoneal administration of hypertonic solutions. He has remarked, "Plasma water volume was strikingly protected by the interstitial portion of available water under conditions of moderate and advanced dehydration and hydration" (2, p. 437).

In the experiment here reported an evaluation was made of the volume change in plasma water attending rather severe water privation in the rat. A test of plasma specific gravity was employed as an index of this change.

METHOD. The experiment used 20 male albino rats of the Sherman strain. Ages ranged from 118–140 days. The animals were maintained on an ad libitum regimen of Purina Laboratory Checkers. The laboratory room housing the animals was kept at a temperature of 70° to 75° Fahrenheit. Relative humidity fluctuated irregularly between 41 per cent and 54 per cent. In the determination of specific gravities, an Eimer and Amend falling drop apparatus was employed.

To reduce emotional resistance, all animals were handled daily for several weeks prior to the experiment. Twelve rats were designated as an experimental group and were deprived of water for 48 hours. Ad lib. feeding was permitted this group during the privation interval. No measurements of food intake were made. Strominger (7) has shown that the food intake of the rat is greatly reduced in the absence of water. Gross observations indicated that our animals consumed very little food during the water privation interval. Immediately prior to privation, weights ranged from 195 grams to 274 grams with a mean value of 240 grams. During privation a 12 per cent weight loss was suffered. The mean weight dropped to 212 grams.

Eight other rats served as a control group. During the 48 hr. interval they were maintained on an ad lib. feeding and *drinking* schedule. The mean weight of this group rose from 259 grams to 262 grams.

[1] This research was supported in full by a grant from the Research Committee of the University of Alabama. The authors also wish to acknowledge the technical assistance of Dr. Dorothy A. Ehmke and the facilities of the Research Laboratory of Human Nutrition of the School of Home Economics, University, Alabama.

Following the privation interval, a blood sample was obtained from each animal by way of heart puncture. Four hours before taking the blood sample, each animal was heparinized.[2] An injection of approximately 450 units dissolved in $\frac{1}{2}$ cc. of isotonic saline was given intraperitoneally.

Surgical anesthesia was effected with nembutal. Fifteen milligrams in $\frac{1}{2}$ cc. of physiological salt solution was administered intraperitoneally and 15 minutes' action was permitted. With the disappearance of defence reflexes, an incision was made in the thoracic region, the ribs and diaphragm sectioned on the left side and the heart exposed.[3] Blood was withdrawn into a 10 ml. syringe through a $\frac{3}{4}$ inch 20 gauge needle. The sample was discharged slowly into a Wintrobe tube which had been previously chilled and evaporated to dryness. Some hemolysis occurred during this operation.

TABLE 1

The effect of water privation on plasma specific gravity

WATER-DEPRIVED ANIMALS		CONTROL ANIMALS	
Rat no.	Specific gravity	Rat no.	Specific gravity
1	1.0289	1	1.0281
2	1.0289	2	1.0249
3	1.0309	3	1.0253
4	1.0323	4	1.0258
5	1.0306	5	1.0255
6	1.0306	6	1.0269
7	1.0312	7	1.0260
8	1.0303	8	1.0273
9	1.0316		
10	1.0320		
11	1.0293		
12	1.0327		
Mean	1.0308	Mean	1.0262
S.D.	0.001276	S.D.	0.001101

Each tube was stoppered tightly and kept at approximately 5°C. until centrifugation. Precautions were taken to cleanse thoroughly the syringe and needle before each heart puncture. A final rinse of distilled water containing a small quantity of heparin (approximately 1575 units per liter) was used. All samples were centrifuged for $\frac{1}{2}$ hour at 5000 r.p.m. The supernatant fluid was removed with a pipette to a clean, dry test tube and the specific gravity determined utilizing the falling drop method described by Barbour and Hamilton (1).

[2] The preparation of the Connaught Laboratories was used (assay, 110 units per mgm.).

[3] This technique sacrifices the rat in the interest of obtaining a large blood sample. A large volume of blood is unnecessary for specific gravity determinations. These samples were employed for other purposes as well.

RESULTS. The specific gravity of each plasma sample is presented in table 1. Means and standard deviations are also given.

DISCUSSION. From table 1, the mean specific gravity for the water-deprived animals is 1.0308. The mean value for the control animals is 1.0262. A consideration of the two distributions reveals no overlap, i.e., the lowest specific gravity obtained for a water-deprived animal is greater than the highest specific gravity obtained for a control animal.

To determine the statistical significance of the difference between the two means, an analysis of variance was performed on the data (Snedecor, 6). The results are summarized in table 2.

The ratio of between-group variance to within-group variance (F value) is 67.76. An F value of 8.28 is significant at the one per cent level of confidence (Snedecor, 6, p. 223). Our obtained value greatly exceeds this figure. We can safely conclude that this difference did not arise by chance, i.e., the probabilities of obtaining such a difference by chance are considerably less than 0.01.

This increase in plasma protein concentration following a 48 hour water privation interval suggests a marked reduction in plasma water volume. Our results agree with the conclusions of Rose, Stucky, Mendel, and Cowgill (5).

TABLE 2

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE
Total	19	12,577.00	
Between groups	1	9,937.25	9,937.25
Within groups	18	2,639.75	146.65

Hamlin and Gregersen (3) obtained a plasma volume increase with the intravenous injection of sodium pentobarbital in the cat. Similarly, Jarcho (4) has reported a decrease in plasma protein concentration in both the cat and the dog under influence of nembutal. He has inferred that hemodilution is effected by entering body fluids. Although phylogenetic differences in blood response to drugs is common, there exists the strong possibility that all of our plasma samples have been to some extent diluted by the action of the anesthesia employed. That is to say, nembutal may exert an effect on the rat similar to that obtaining in the instance of the cat and the dog. For this reason the absolute specific gravities here reported have no essential validity.

SUMMARY

Specific gravity determinations were made upon the plasma samples of water-deprived rats and upon plasma samples of rats maintained on an ad libitum drinking regimen. The mean specific gravity of the former group was found to be significantly greater. It was concluded that a decrement in plasma water volume occurs during severe water privation in the rat.

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ON THE ENERGY-RICH PHOSPHATE SUPPLY OF THE FAILING HEART[1]

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From the literature on the metabolism of the failing mammalian heart the impression may be gained that myocardial failure is associated with a deficiency in adenosine triphosphate (ATP) and phosphocreatine, the compounds which constitute the direct source and the nearest reservoir of chemical energy for muscular contraction. Thus, according to Cruikshank (1), the ratio of the phosphate of phosphocreatine over inorganic phosphate is an index of the physiological state of the heart. This contention, however, is based on the behavior of hearts deprived of oxygen or nutrients and hence lacks validity as a generalization. Herrmann and Decherd (2) and Myers (3), who observed losses in total creatine in failing human hearts, imply that the phosphocreatine content probably was correspondingly diminished. Accompanying losses in oxypurines and total acid-soluble purines have been accepted as a probable indication of a decrease in ATP (3, 4). On evidence presented below, the assumption of a proportionality between total and phosphorylated creatine in cardiac muscle must be questioned. Whether the concentrations of total acid-soluble purines and of oxypurines are proportional to the concentration of ATP remains to be determined.

In view of the limitations of the above studies, it seemed desirable to examine the relation between cardiac function and energy-rich phosphate supply under conditions *a*, where the functional state of the heart could be described in quantitative terms; *b*, where its content of labile phosphates could be determined directly, and *c*, where there was reasonable assurance that adequate oxygenation and nutrition of the myocardium was not interfered with by non-experimental factors. The heart-lung preparation perfused with a large volume of blood was selected as a suitable test object.

METHODS. *Cardiodynamic measurements.* Healthy male and non-pregnant female dogs in the post-absorptive state, weighing between 8 and 12 kgm., were used. The Starling heart-lung preparation (HLP) was made with the animals under pentobarbital anesthesia. Large dogs anesthetized with ether served as blood donors. The blood was defibrinated and used either immediately or stored at 2°C. for one to four days. Differences in the results attributable to the length of storage were not noticed. The total amount of circulating blood in the heart-lung system was about 900 cc. at the beginning of the experiments. Its temperature was kept constant at about 38°C. Arterial resistance was maintained at 80 mm. mercury. The level of the blood in the venous supply reservoir (inflow level) was held constant at approximately 110 mm. above the opening of

[1] This work was supported by a grant from the Life Insurance Medical Research Fund.

the inferior vena cava, except for brief tests of myocardial competence described below.

Myocardial failure either developed spontaneously after several hours or was induced by the administration of one of a number of drugs with a negative inotropic action. These drugs were: the general anesthetics and anticonvulsants' pentobarbital, chlorobutanol, paraldehyde, diphenylhydantoin, and propazone (5,5-di-n-propyl-oxazolidinedione-2,4); the local anesthetics procaine and tetracaine; the antimalarial quinacrine; and the organic mercurial diuretic mersalyl. Preparations of competent hearts served as controls.

The following measurements of cardiac and circulatory function were taken by methods previously described (5): systemic output, systemic arterial pressure, pulmonary arterial pressure, and, in a number of experiments, left atrial pressure. Right atrial pressure was measured with a water manometer connected to the inferior vena cava. Coronary sinus outflow was channeled into a Morawitz cannula and measured with a graduated tube and stop watch or with a Condon flowmeter. The value thus obtained was taken to be 60 per cent of the total coronary flow. The heart rate was determined with a stop watch. The work performed by the heart was calculated from the systemic and pulmonary arterial pressures and the output of both ventricles, acceleration work being neglected. The reserve capacity of the heart for performing work, or, more specifically, its competence to take care of an increase in blood supply, was estimated according to Krayer (6) from the response of the right atrial pressure to an increase in the height of the inflow level. The results of these tests of cardiac competence have been expressed as the "competence index" (C.I.). This index is defined as follows:

$$\text{C. I.} = \frac{\text{increase in inflow level} - \text{increase in right atrial pressure}}{\text{increase in inflow level}}$$

It ranges from 0 for a fully incompetent heart to 1 for a fully competent heart. In the present experiments the increase in right atrial pressure (in millimeters water) was determined following an increase in the inflow level of 50 mm. Thus, the competence index was always computed:

$$\text{C. I.} = \frac{50 - \text{increase in right atrial pressure}}{50}$$

Chemical determinations. Five minutes following the last competence test and immediately following the last set of the other measurements, a sample of heart muscle from the apical region of the left ventricle, weighing 3 to 5 grams, was taken for analysis. It was quickly severed with a sharp razor and dropped immediately into a Dewar flask filled to the rim with liquid nitrogen. Not more than one second elapsed between severance of the apical piece from the rest of the heart and immersion into the liquid.

The completely frozen sample was rapidly weighed on a torsion balance and transferred quickly into a chilled porcelain mortar in which it was ground with 7 to 8 volumes of ice-cold 5 per cent trichloroacetic acid to a fine suspension. The

suspension was left standing for two minutes and the solid particles were filtered off on phosphate-free filter paper and washed with another 5 volumes of trichloroacetic acid. The filtrate was made just alkaline to phenolphthalein with a small volume of 8 N sodium hydroxide. This whole procedure was carried out in the cold room under ice cooling.

The neutralized trichloroacetic acid filtrate was analyzed for total acid-soluble phosphate (TASP), free inorganic phosphate, the phosphate of phosphocreatine, and the labile phosphate of adenosine polyphosphate (APP). In hearts excised several minutes after death (7) and also in aerated heart muscle slices (8) the last-named phosphate fraction contains some adenosine diphosphate. In the fresh heart *in situ*, on the other hand, probably all the detectable labile nucleotide phosphate is that of ATP (9). However, since this has not yet been shown to be the case also in the fresh isolated heart, the designation labile P of APP rather than labile P of ATP has been given to the easily hydrolyzable nucleotide phosphate of the HLP hearts.

The final determinations of phosphate in the orthoinorganic form were made simultaneously by the method of Fiske and Subbarow (10) with a visual colorimeter. For the determination of TASP an aliquot of the filtrate was ashed with sulfuric and nitric acids (10). The material was then diluted with water and heated in a boiling water bath in order to decompose any pyrophosphate or metaphosphate formed during the ashing. Inorganic phosphate was determined directly after precipitation as the calcium salt (11). The phosphate of phosphocreatine was estimated by subtracting the inorganic phosphate from the value obtained after 30 minutes of acid hydrolysis at room temperature in the presence of molybdate (11). The labile P of APP was estimated by subtracting the latter value from that obtained after hydrolysis with 1 N hydrochloric acid for 7 minutes at 100°C. (12).

In a few experiments determinations of acyl phosphate were carried out. A 3 cc. aliquot of unneutralized trichloroacetic acid filtrate was used for this purpose. The specific procedure of Lipmann and Tuttle (13) was followed with slight modifications suggested by Doctor Lipmann.

During the course of the study it became desirable to check the identity of the phosphate fraction analyzed as the phosphate of phosphocreatine. This was accomplished in two ways: *a*. The speed of hydrolysis of phosphocreatine was measured. For this purpose the reaction was carried out under mild conditions according to Lowry and Lopez (14), who use ascorbic acid at pH 4 as reducing agent in the determination of phosphate. The concentration of ammonium molybdate was 0.25 per cent. The course of the reaction was followed with a photoelectric colorimeter, readings being taken every two minutes, and the half-life was computed. *b*. Phosphocreatine was determined as creatine. The compound was precipitated in the manner described by Fiske and Subbarow (11) by treating 3 cc. of neutralized filtrate with a corresponding amount of copper acetate at a pH between 7 and 8. The precipitate was centrifuged off and washed with a few cubic centimeters of water. The combined supernatant and washing contained the free creatine of the sample. Creatinine,

in the small amounts present, remained likewise in the supernatant. The two compounds were estimated according to Folin and Wu (15). Total creatine plus creatinine were estimated in a separate sample of the filtrate. The difference between the two results was taken to represent phosphorylated creatine.

RESULTS. The main results of this study are given in table 1. They are grouped into three sections, representing the normal controls, the spontaneously failing hearts, and the hearts made to fail by the cardiotoxic drugs. Although the various drugs may not impair the contractility of the heart by the same mechanism, there is some justification in grouping together the results obtained with these drugs, inasmuch as both the cardiodynamic and the chemical changes were, on the whole, in the same direction. The significance of the differences between the means of the two groups of failing hearts on the one hand and the controls on the other hand is evaluated statistically.

The cardiodynamic data in table 1 illustrate the impaired activity of the hearts in the two experimental groups at the time the cardiac muscle sample was removed for analysis. A markedly elevated right atrial pressure signalizes right ventricular failure. Left atrial pressure was greatly elevated in all the spontaneously failing preparations in which it was measured, showing that failure was left- as well as right-sided. A sharp rise in left atrial pressure was observed also in a heart poisoned with mersalyl. In the experiments with the other cardiotoxic drugs, left atrial pressure was not recorded. Most of these drugs, however, have been tested previously in this laboratory on the HLP and were found to be as potent in causing increases in the left as in the right atrial pressure. The reserve capacity of the failing hearts, reflected in the C. I., was largely used up or was completely exhausted. Actual work performance was greatly diminished, due principally to a sharp decline in minute output. This decline was entirely the result of a severe reduction in systemic output. Some hearts were weakened to such an extent that no blood or only negligible amounts were being pumped into the system. Coronary flow, in spite of a decreased arterial pressure, was increased in most cases, especially in spontaneous failure and in failure induced by chlorobutanol and diphenylhydantoin. In the majority of the experiments, more blood was circulating through the coronaries at the time of the terminal measurements than through the system. The failing hearts remained to the end adequately supplied with blood.

The heart rate was decreased in every instance of spontaneous failure. All compounds with a negative inotropic action, except mersalyl, had also a negative chronotropic action. The local anesthetics produced, in addition, irregularities of rhythm.

Table 1 shows that loss of myocardial contractility was not associated with a loss in APP and phosphocreatine, but, on the contrary, was accompanied in most instances by a gain in phosphocreatine. The discrepancy between the cardiodynamic and chemical data is striking. In spite of extreme differences in performance and functional capacity, the APP concentration remained relatively constant. Lowered values were found in a few failing hearts which also were correspondingly poorer in TASP, probably because of the presence of edema

fluid or of comparatively large amounts of coronary blood in the tissue. Otherwise, the TASP concentration was likewise undiminished in failure. The concentration of phosphocreatine, which in the control group ranged from 12.0 to 17.4 mgm. P per cent, with an average of 14.3, was significantly higher in the experimental groups. In spontaneous failure it varied from 15.3 to 22.7 mgm. P per cent, averaging 19.5; in drug-induced failure the range was 12.4 to 28.4, the average 18.0. The inorganic phosphate was somewhat diminished. In the two very feebly contracting hearts at the bottom of the list, the phosphocreatine concentration, amounting to 22.7 and 20.0 mgm. P per cent, was among the highest in the series. It should be noted that even in these two cases of extreme failure, in which the systemic circulation had ceased, the coronary circulation was still functioning. Although diminished to a small fraction of the normal volume, it must have been sufficient, in view of the negligible amount of work performed, to maintain metabolism. The high levels of APP and phosphocreatine are a good proof of this contention.

Determinations of acyl phosphate were performed only in a few scattered experiments. However, the results show clearly enough (table 1) that the concentration of this energy-rich phosphate fraction is practically negligible in the failing as well as in the non-failing heart.

It is evident from the results in table 1 that, under the conditions prevailing in the present experiments, myocardial failure developing spontaneously or precipitated by the various pharmacological agents used is not due to exhaustion of the immediately available supply of energy for systole (or for diastole).

Identity of the "excess" phosphocreatine of the isolated heart. The values of phosphocreatine in table 1 fall within the same range as those obtained by Pollack, Flock, Essex, and Bollman (17) in the heart of the dog HLP. These authors drew attention to the observation that isolated dog hearts are frequently richer in phosphocreatine than is the heart in the intact animal. We have made phosphate determinations in the hearts of six intact anesthesized dogs kept under artificial respiration and found, in agreement with Pollack *et al.*, the phosphocreatine content to be considerably below that of the normal HLP heart. The values for the six hearts ranged from 7.0 to 11.4 mgm. P per 100 grams apex, averaging 8.9 mgm. The distribution of the other organic acid-soluble phosphate fractions, including acyl phosphate, was about the same as in the heart of the HLP.

Before accepting as a fact that the isolated heart has a greater phosphocreatine reserve than the heart *in situ*, it had to be made reasonably certain that the bulk of the phosphate analyzed as that of phosphocreatine actually came from phosphocreatine and was not derived in part from some other phosphate fraction of similar analytic behavior. For this purpose the identity of the phosphate of the HLP heart determined as the phosphate of phosphocreatine by the method of Fiske and Subbarow was subjected to verification by the use of the procedures outlined above, i.e., by measuring the half-time of splitting phosphocreatine under the mild hydrolytic conditions specified by Lowry and Lopez (14), and by determining the compound as creatine. Three non-failing and four spontaneously failing hearts were used for this purpose.

TABLE 1

Acid-soluble phosphates in normal and failing hearts of dog heart-lung preparations

DOG NO.	CAUSE OF HEART FAILURE	DOSE	TIME OF SURVIVAL	VENTRICULAR RATE	MEAN SYSTEMIC ARTERIAL PRESSURE	PULMON. ARTERIAL PRESSURE	RIGHT ATRIAL PRESSURE	LEFT ATRIAL PRESSURE	SYSTEMIC OUTPUT	CORONARY FLOW	TOTAL OUTPUT	WORK	C.I.	P, MGM. PER 100 GRAM APEX				
														Inorganic phosphate	Phosphocreatine	Labile P of APP	Acyl phosphate	TASP
		mg.	*min.*	*per min.*	*mm. Hg*	*mm. H_2O*	*mm. H_2O*	*mm. H_2O*	*cc./min.*	*cc./min.*	*cc./min.*	*kg-m/min.*						
20	Normal heart		39	136	111	187	9		460	55	515	0.870	0.95	*28.9*	*14.1*	*37.4*		*107.2*
22	Normal heart		37	166	105	174	29		430	55	485	0.772	0.94	*34.8*	*13.3*	*39.8*		*115.0*
16	Normal heart		54	152	109	183	21		441	50	491	0.814	0.92	*25.7*	*14.7*	*33.4*		*95.8*
59	Normal heart		163	124	97	175	31	50	238	167	405	0.603	0.92	*26.8*	*12.0*	*31.5*	*0.8*	*100.2*
87	Normal heart		74	152	107	205	28	57	516	50	566	0.940	0.90	*29.9*	*13.6*	*35.4*	*0.4*	*105.2*
75	Normal heart		123	128	100	233	55		367	131	498	0.794	0.80	*22.7*	*15.7*	*33.7*		*102.2*
83	Normal heart		102	134	100	153	58	71	394	128	522	0.790	0.78	*28.8*	*13.8*	*33.9*		*97.4*
86	Normal heart		70	130	103	270	52	79	460	39	499	0.834	0.71	*27.6*	*17.4*	*36.6*		*109.8*
Mean			82.6	140.3	104.0	197.5	35.4	64.3	413.3	84.4	497.7	0.8021	0.865	*28.15*	*14.33*	*35.21*		*104.10*
±S.E.				±5.2	±1.7	±13.3	±6.2	±6.6	±29.6	±17.5	±16.0	±0.0342	±0.031	*±1.24*	*±.58*	*±.94*		*±2.29*
79	Spontaneous deterioration		245	140	101	193	69	151	136	111	247	0.439	0.29	*27.4*	*18.4*	*31.2*		*110.2*
84	Spontaneous deterioration		322	92	96	386	84		93	266	359	0.604	0.28	*20.5*	*22.5*	*31.5*		*99.3*
85	Spontaneous deterioration		275	104	62	300	111	152	14	106	120	0.137	0.27	*29.1*	*22.7*	*34.7*		*116.4*
12	Spontaneous deterioration		243	132	113	335	57		280	161	441	0.819	0.26	*20.0*	*16.7*	*26.0*		*89.3*
95	Spontaneous deterioration		247	112	79	224	93	171	60	250	310	0.399	0.20	*24.0*	*15.3*	*28.1*		*94.7*
80	Spontaneous deterioration		292	104	92	268	74		6	128	134	0.203	0.16	*24.1*	*22.2*	*33.2*		*108.0*
27	Spontaneous deterioration		115	124	102	251	72		342	56	388	0.647	0.14	*28.2*	*20.4*	*41.1*	*0.0*	*101.7*
82	Spontaneous deteroiration		258	130	87	236	90	173	100	217	317	0.447	0.13	*20.3*	*18.0*	*25.5*	*1.0*	*93.2*
Mean			249.6	117.3†	91.5	274.1†	81.3*	161.8*	128.9*	161.9	290.8*	0.4619*	0.216*	*24.20*	*19.53**	*31.41*		*101.60*
± S.E.				±5.9	±5.6	±22.4	±6.0	±5.9	±13.6	±26.6	±41.1	±0.0801	±0.024	*±1.32*	*±1.00*	*±1.81*		*±3.30*

10	Procaine HCl	250	198	130‡	91	230	80		120	189	309	0.450	0.32	*23.9*	*15.7*	*31.4*		
52	Propazone Na	2650	210	110	79	202	81		66	139	205	0.259	0.26	*20.2*	*15.4*	*33.4*		*96.4*
19	Tetracaine HCl	30	180	80‡	80	285	82		280	52	332	0.452	0.14	*17.2*	*28.4*	*37.4*		*108.1*
18	Tetracaine HCl	40	88	92‡	91	216	89		127	84	211	0.304	0.11	*19.2*	*17.8*	*30.0*		*102.0*
92	Mersalyl	187	213	168	95	273	89	229	99	49	148	0.250	0.06	*30.1*	*17.4*	*32.7*		*108.1*
34	Quinacrine Di-HCl	20	141	128	83	226	116		25	52	77	0.103	0.02	*26.1*	*16.1*	*38.9*	*1.1*	*107.2*
11	Procaine HCl	400	115	80‡	101	321	76		260	77	337	0.568	0.00	*23.4*	*18.6*	*33.0*		
21	Diphenylhydantoin Na	140	125	110	91	245	85		114	228	342	0.500	0.00	*21.8*	*12.4*	*27.5*		*89.3*
35	Chlorobutanol	310	182	114	82	255	91		52	266	318	0.433	0.00	*21.6*	*14.9*	*28.0*	*0.5*	*101.3*
9	Paraldehyde	3960	174	108	49	198	122		0	64	64	0.055	0.00	*17.6*	*17.1*	*32.0*		
56	Quinacrine Di-HCl	70	211	24	20	132	127		0	12	12	0.005	0.00	*20.3*	*22.7*	*37.2*		*115.1*
60	Pentobarbital Na	300	187	112	14	106	126		0	13	13	0.004	0.00	*23.8*	*20.0*	*31.0*		*105.1*
Mean			168.7	104.7*	73.0*	224.1	97.0*		95.3*	102.1	197.4*	0.2819*	0.076*	*22.10**	*18.04*†	*32.71*		*103.62*
± S.E.				±10.0	±8.4	±17.5	±5.8		±27.3	±24.3	±38.0	±0.0583	±0.032	*±1.06*	*±1.21*	*±1.04*		*±2.51*

* Probability <0.01 and

† Probability between 0.01 and 0.02, as indicated by the t-test (16), that the samples were drawn from the same population as the normal hearts.

‡ Irregular rhythm.

The results of these experiments are shown in table 2. The phosphocreatine content of the seven hearts as determined by the method of Fiske and Subbarow ranged from 13.8 to 22.7 mgm. P per cent. The method of Lowry and Lopez yielded values close to these. The half-life of hydrolysis was 14 and 28 minutes in two of the experiments; in the other four experiments it was close to 20 minutes; 20 minutes, according to Lowry and Lopez, is the half-life of the reaction in 0.25 per cent ammonium molybdate at pH 4. Thus, the phosphate fraction determined as phosphate of phosphocreatine behaved, on the whole, like true phosphocreatine.

The results of the creatine determinations in table 2 reveal, first of all, in confirmation of earlier data reported by Pollack *et al.* (17), a lack of correlation between the amounts of phosphorylated and of total creatine in the cardiac muscle of the dog HLP. The creatine bound to phosphate, computed from the phosphocreatine values obtained by the Fiske-Subbarow method, varied from 21 to 41 per cent of the total creatine. In the 25 experiments of Pollack *et al.* it varied, according to calculations made from their data, from 14 to 54 per cent. The coefficient of correlation between the amount of phosphocreatine and of total creatine, based on the combined data of Pollack *et al.* and of table 2, has the very low value of 0.08. The fact that in cardiac muscle only a relatively small fraction of the total creatine is combined with phosphate helps explain why this correlation is so low.

TABLE 2

Phosphocreatine in the heart of the heart-lung preparation of the dog as determined by various methods

DOG NO.	CONDITION OF HEART	P OF PHOSPHOCREATINE		$t_{\frac{1}{2}}$ OF PHOSPHOCREATINE HYDROLYSIS*	TOTAL CREATINE†	CREATINE OF PHOSPHOCREATINE	MOLAR RATIO OF CREATINE OF PHOSPHOCREATINE TO PHOSPHATE OF PHOSPHOCREATINE (FISKE-SUBBAROW)
		Fiske-Subbarow	Lowry-Lopez				
		mgm. %	*mgm. %*	*min.*	*mgm. %*	*mgm. %*	
83	Normal	13.8	14.1	14	275	58	1.02
86	Normal	17.4	15.6	21	209	55	0.78
87	Normal	13.6	14.0	23	207	72	1.28
82	Spontaneous failure	18.0			281	80	1.09
84	Spontaneous failure	22.5	19.4	16	252	72	0.78
85	Spontaneous failure	22.7	20.2	28	228	79	0.85
95	Spontaneous failure	15.3	14.8	18	172	55	0.87

* In 0.25 per cent ammonium molybdate at pH 4 and room temperature.

† Includes preformed creatinine.

The creatine fractionation yielded ratios of moles of creatine of phosphocreatine to moles of phosphate of phosphocreatine (Fiske-Subbarow values) varying from 0.78 to 1.28. In the two hearts with the highest values of P of phosphocreatine (22.5 and 22.7 mgm. per cent) the ratios were 0.78 and 0.85. A 1:1 ratio, based on the figures for bound creatine, would have been obtained for P of phosphocreatine values of 17.6 and 19.3 mgm. per cent. These are con-

centrations which still are twice as high as those in the heart muscle of the intact dog. From these data it appears that the bulk of the phosphate of the dog HLP heart analyzed as the phosphate of phosphocreatine actually is combined with creatine.

Together with the finding that the highly labile phosphate of acetyl phosphate or other acyl phosphates is uniformly very low both in the isolated heart and in the heart *in situ*, the results presented in tables 1 and 2 leave little doubt that the heart of the HLP is considerably richer in phosphocreatine than the heart of the intact animal.

The cause of the elevated phosphocreatine content of the HLP heart has yet to be ascertained. Pollack *et al.* (17) have merely commented that the phenomenon is not simply a restoration of something broken down during the setting up of the HLP. Preliminary experiments suggest that exclusion of the liver from the circulation may produce an increase in the phosphocreatine content of the heart.

Relation between the phosphocreatine concentration and heart rate, work performance, and survival time of the heart-lung preparation. Inspection of table 1 reveals three variables which may be singled out, both on the basis of theoretical considerations and on the basis of the actual results, as possibly having contributed to changes in the phosphocreatine level of the HLP hearts. These variables are heart rate, work performance, and survival time of the preparation (measured from the moment the heart-lung circuit was established). It was seen from table 1 that hearts beating at low frequencies and doing little work tend to accumulate a high phosphocreatine reserve. Lowering of heart rate and of work performance are, in turn, consequences of increasing survival time, at least in preparations not treated with toxic drugs. The importance of the time factor in causing elevation of the phosphocreatine level in the heart of the HLP has been stressed by Pollack *et al.* (17). These authors, however, gave no data on heart rate and work performance.

The extent to which the phosphocreatine concentration is correlated to heart rate, to work, and to the survival time of the HLP is shown in table 3. Product-moment correlation coefficients were calculated from the results of the 28 experiments in table 1, assuming that the depressant drugs have no direct specific action on the phosphocreatine content. The values obtained indicate the existence of significant over-all relationships between the amount of phosphocreatine on the one hand, and heart rate, work, and survival time on the other hand. The respective zero order r's are -0.58, -0.47, and $+0.48$. However, since rate and work of the HLP usually decrease with time, the zero order correlations between rate and phosphocreatine and between work and phosphocreatine may be high on account of the common influence of the time factor, and the latter may correlate significantly with phosphocreatine by virtue of its influence on rate and work.

A truer estimate of the pure relations between phophocreatine and the other three variables than that provided by the zero order correlations is given by the partial correlation coefficients in table 3. It is seen that when the survival time

of the HLP is statistically kept constant, the coefficient of correlation between phosphocreatine and rate is only moderately reduced to the still fairly sizable value of −0.51. On the other hand, keeping the survival time constant reduces the correlation coefficient for phosphocreatine and work from −0.47 to −0.29. Similarly, removal of the influence of rate and work on the relation between phosphocreatine and survival time lowers the coefficient from 0.48 to 0.31. Of the three partial correlation coefficients, only that linking phosphocreatine and heart rate can be considered to be significant.

This analysis of the results suggests that among all the cardiodynamic variables measured, the heart rate is by far the most important in determining the phosphocreatine level of the heart.[2] The direct proof of this contention remains to be

TABLE 3

Coefficients of correlation between phosphocreatine in the heart of the dog heart-lung preparation and heart rate, work performance, and time of survival of the preparation

(1) Phosphocreatine; (2) heart rate; (3) work; (4) time of survival.

ZERO ORDER r*	ATTAINED LEVEL OF SIGNIFICANCE‡	PARTIAL r†	ATTAINED LEVEL OF SIGNIFICANCE‡
	P		P
$r_{12} = -.58$	0.01	$r_{12.4} = -0.51$	0.01
$r_{13} = -.47$	0.02	$r_{13.4} = -0.29$	0.2
$r_{14} = +.48$	0.01	$r_{14.23} = +0.31$	0.2

* $r = \dfrac{\Sigma xy}{\sqrt{\Sigma x^2 \cdot \Sigma y^2}}$, where x and y are the deviations of the individual values of the two variables from their respective means.

† Calculated by the general formulae:

$$r_{ab \cdot c} = \frac{r_{ab} - r_{ac} \cdot r_{bc}}{\sqrt{(1 - r_{ac}^2)(1 - r_{bc}^2)}}$$

and

$$r_{ab \cdot cd} = \frac{r_{ab \cdot d} - r_{ac \cdot d} \cdot r_{bc \cdot d}}{\sqrt{(1 - r_{ac \cdot d}^2)(1 - r_{bc \cdot d}^2)}}$$

‡ As indicated by the t-test (16)

furnished by actual experiment. There can be little doubt, however, that a major reason for the elevated phosphocreatine content of the failing hearts in table 1 was their lowered rate of beat.

DISCUSSION. The present experiments have demonstrated that myocardial failure can occur in the presence of an adequate supply of energy for the cardiac cycle. Specifically, it has been shown that in hearts failing spontaneously or as a result of the toxic action of general anesthetics, local anesthetics, quinacrine, or mersalyl, there is no deficiency in APP and phosphocreatine. Since the ultimate goal of metabolic energy production in muscle is to supply energy utilizable for contraction in the form of the energy-rich phosphate bonds of APP and,

[2] The difference between the phosphocreatine content of the isolated heart and of the heart *in situ* is not due to a difference in heart rate.

in the second place, of phosphocreatine, it is clear at once that these types of myocardial failure are not caused by impairment of energy metabolism. On the contrary, the energy reserves for contraction are more abundant than in the well-functioning heart, as witnessed by the unusually large phosphocreatine stores. It is, therefore, the utilization and not the generation of phosphate bond energy that is at fault.

This concept of a mechanism of experimental myocardial failure is in agreement with the work of Visscher (18), who concluded, from measurements of work and oxygen consumption, that the defect underlying the spontaneous failure of the isolated mammalian heart perfused with blood is one of energy utilization and not of energy production. It is incompatible with the view of Katz (19), likewise based on measurements of work and oxygen consumption, that this type of cardiac failure is due to impairment of energy metabolism.

Burns and Cruikshank (20) observed that, in the ventricles of dog HLP's exposed to the combined action of insulin and epinephrine, the APP and phosphocreatine were reduced by 50 and 25 per cent, respectively, below the values found in the ventricles of intact dogs. Their implication that these changes are representative of the behavior of the mammalian heart fatigued in the presence of oxygen appears hardly warranted. Their further finding that the heart of the normal HLP may contain less phosphocreatine than the heart of the intact animal is in sharp disagreement with the data of Pollack *et al.* (17) and of the present study. Weicker (21) reported that he found the APP and phosphocreatine content of isolated cat hearts to be greatly reduced in insufficiency following long-lasting perfusion. The fact that the perfusion fluid used was Tyrode solution and not blood may account for the depletion of the energy-rich phosphates. It is not unlikely that during the course of the experiments the oxygen supply to the myocardium became a limiting factor in the synthesis of these compounds. The isolated frog heart, which usually is kept at a much lower temperature than mammalian heart preparations and hence requires comparatively less oxygen, will beat, according to Clark *et al.* (22), for many hours in oxygenated Ringer solution with only insignificant or moderate losses of phosphocreatine and APP. Clark *et al.* (cf. 22) have also shown that the frog heart can be weakened by a general anesthetic (ethyl urethane) and by other pharmacological means without a decrease in its phosphocreatine content. Of interest in connection with these and with the present findings is the observation of Jokl (23) that in rats forced to run to exhaustion, the phosphocreatine concentration in the fatigued muscles of the thigh rises to abnormally high values.

In view of the poor correlation found to exist between total creatine and phosphocreatine in the isolated heart, it seems hardly justified to assume the existence of a high correlation in the heart *in situ.* Indeed, data presented recently by Davies *et al.* (24) reveal not only a marked difference between atria and ventricles with respect to the percentage of the total creatine present in phosphorylated form, but show also that this percentage varies considerably among atria and among ventricles of animals of the same species. On the basis of existing evidence, therefore, it is difficult to accept the inference of Herrmann and Decherd (2) and of Myers (3) that the loss in total creatine which they observed in failing

human hearts probably reflects a loss in phosphocreatine. It is, of course, not improbable that in congestive heart failure in the human and in the intact animal, where, due to cardiac hypertrophy and other factors, the oxygen supply to the myocardium is in all likelihood poorer than in the failing HLP, the concentrations of phosphocreatine and of APP may be low. From the relation between cardiac rate and phosphocreatine content found in the present experiments, a low level of phosphocreatine and possibly of APP is to be expected if congestive heart failure is accompanied, as is very often the case, by tachycardia. It is regrettable that none of the authors cited above has published any information on the rate at which the hearts which they analyzed had been beating.

In a parallel study dealing with metabolic aspects of the toxic action of the general and the local anesthetics on the heart (25) it was found that the former group markedly inhibits the respiration of slices of dog heart muscle in concentrations which reduce, but do not abolish, the contractility and automaticity of the organ. This finding suggested that the negative inotropic and negative chronotropic action of these drugs may be due to a depression of respiratory metabolism. The present finding that hearts depressed by such general anesthetics as pentobarbital, chlorobutanol, paraldehyde, and propazone are able to maintain a normal supply of APP and phosphocreatine speaks strongly against this notion. Since in the present experiments the hearts were exposed to these drugs for a period of one-half hour or more before a sample was taken for analysis, it appears highly improbable that the energy for the synthesis of APP and phosphocreatine was furnished anaerobically. Therefore, unless one assumes that the activity respiration of the heart is less sensitive to general anesthetics than is the respiration of quiescent cardiac slices, the differential action of these drugs on respiratory activity and on energy-rich phosphate supply may be explained on the ground that the energy demands of the weakened hearts were so low that they could be satisfied even by a markedly depressed respiration.

A corollary of the present findings concerns the mechanism of action of compounds capable of relieving the types of myocardial failure investigated in this study. The cardiac glycosides and epinephrine, for example, are known to restore the contractile power of the heart in spontaneous and in barbiturate failure and have been observed in this laboratory to act in the same way in cardiac failure precipitated by other general anesthetics and by local anesthetics, mercurials, and quinacrine. Inasmuch as none of these failures is caused by impairment of energy metabolism, the positive inotropic action of the cardiac glycosides, of epinephrine, or of any other drug effective in these failures, cannot be the result either of effects on energy metabolism, regardless of what metabolic changes may be produced in the myocardium. The primary action of these substances, like that of the negative inotropic agents studied and like the changes leading to spontaneous heart failure, must be concerned with a phase of myocardial contraction other than the generation of utilizable chemical energy.

SUMMARY

Hearts of dog heart-lung preparations failing spontaneously or as a result of the administration of various general anesthetics or local anesthetics, or of

mersalyl or quinacrine, have a normal supply of adenosine polyphosphate and are richer in phosphocreatine than non-failing hearts. Both non-failing and failing hearts contain only negligible amounts of acyl phosphate.

The elevated phosphocreatine content of the failing hearts can probably be accounted for in part by a lowered rate of beat.

The identity of the phosphate fraction determined as the phosphate of phosphocreatine has been verified. It has been confirmed that in the heart of the heart-lung preparation, non-failing as well as failing, this fraction is considerably higher than in the heart *in situ*.

Analysis of earlier and of the present data reveals a lack of good correlation between the amounts of phosphorylated and of total creatine in the heart of the heart-lung preparation of the dog.

The results indicate that the types of myocardial failure studied are due to impairment of the utilization and not of the generation of phosphate bond energy. The implication of this conclusion in regard to the mechanism of the positive inotropic cardiac action of certain pharmacological agents is pointed out.

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DISTRIBUTION OF ESTERASE IN LYMPH FROM VARIOUS REGIONS AND IN RELATION TO LYMPHOID TISSUE[1]

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Dog plasma contains an enzyme in the α_2 globulin fraction capable of hydrolyzing acetylcholine and other esters. The determination of the acetylcholine-splitting activity of plasma is a convenient method of studying this esterase. In a previous report (1) it was shown that considerable plasma esterase exists in the liver, and that this store can be mobilized under certain conditions. In the present paper the esterase content of lymph from the liver and other sources is studied; the influence upon lymph of two procedures, shown in the previous report to affect the distribution of esterase between plasma and liver, is examined; and the possibility that plasma esterase may arise from lymph nodes or lymphocytes is investigated.

METHODS. Healthy male or female mongrel dogs were used after having been kept in the laboratory for two or three days to assure that they were adequately hydrated. The distribution of plasma esterase was disturbed in one group of dogs by the administration by stomach tube, immediately before feeding on each of the two days preceding the collection of lymph, of 1 cc. per kgm. of carbon tetrachloride diluted with an equal volume of Wesson oil. In another group of dogs hemodilution was brought about by the transfusion of concentrated solutions of esterase-free human or bovine[3] crystalline plasma albumin. Samples of blood and lymph were taken 30 to 60 minutes after this transfusion, at a time when the plasma esterase concentration had fallen to about 80 per cent of its normal value, as shown in the previous report (1).

All experiments involving the cannulation of lymphatics were carried out under sodium pentobarbital anesthesia, a uniform level of anesthesia being maintained by the administration of small doses of the drug during the course of the experiment.

Collection of lymph from the thoracic duct and the left cervical duct were accomplished by customary procedures through an incision in the neck (2). Liver lymph and mesenteric lymph were collected at laparotomy (transversalis incision), the former from a cannula inserted in one of the lymphatics which can

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be seen running on the lesser omentum and the neck of the gall bladder (3), and the latter from a large lymph trunk leaving the pancreas of Aselli. In a number of experiments, the dye T-1824 was injected in 0.3 per cent solution into the various lobes of the liver in order to ascertain that the fluid collected actually was liver lymph. In all cases tested, the administration of dye into either the right lateral or the central lobe was followed within a few seconds by the appearance of dye in the liver lymph.

Blood for analysis was collected from the femoral artery of one leg while injections were made into the corresponding vein of the other leg. Dry heparin was added to all lymph samples as an anticoagulant and 0.1 cc. of 1 per cent heparin solution in 0.9 per cent NaCl was used per 5 cc. of blood.

Cholinesterase determinations were performed by the method of Ammon (4); 0.025 M sodium bicarbonate buffer (pH 7.4) in 0.25 M sodium chloride was used as a reaction medium, and 0.08 M acetylcholine bromide (Eastman Kodak Company, once recrystallized) as a substrate. Results are expressed as millimoles of acetylcholine hydrolyzed by 1000 grams of plasma or tissue in one hour.

Total protein determinations were carried out refractometrically and were checked occasionally against the copper sulfate specific gravity method of Phillips et al. (5).

Lymph nodes removed from anesthetized or freshly killed dogs were homogenized in 5 volumes of 0.9 per cent NaCl for the determination of esterase activity. Lymphocytes were obtained from fresh lymph nodes teased apart by needles in physiological saline containing 0.2 per cent dextrose. The resulting suspensions were allowed to stand for 10 minutes to permit settling out of larger fragments of tissues. Lymphocytes were then separated by carefully layering the suspension over isotonic 21 per cent bovine albumin solution (Armour and Co.) and centrifuged at 500 r.p.m. for 5 minutes, then at 2000 r.p.m. ($r = 24$ cm.) for 15 minutes[4]. The layer of colorless cells between the supernatant and the albumin was harvested and found to consist mainly of lymphocytes with some admixture of damaged cells. This mixture was resuspended in saline in a graduated centrifuge tube and centrifuged for 15 minutes. By this procedure it was possible to obtain from 3 to 4 grams of fresh tissue 0.2 to 0.3 cc. of packed cells, mainly lymphocytes.

RESULTS. Table 1 shows the results of analyses of plasma and of lymph from the liver and cervical and thoracic ducts. In normal dogs the esterase activity of blood plasma and of liver lymph are very nearly equal. In confirmation of earlier observations (6) it was found that cervical lymph contains considerably less esterase activity—about 30 per cent that of plasma—while thoracic duct lymph occupies an intermediate position. In liver lymph the ratio of esterase activity to total protein is identical with that of plasma, while in cervical lymph this value is considerably lower. The values for thoracic duct lymph are intermediate.

Liver injury by carbon tetrachloride results in large increases in the blood

[4] The authors are indebted to Dr. C. J. Favour for suggesting this technique.

plasma esterase activity (1). As shown in table 1, liver lymph remains similar to plasma. The composition of cervical lymph tends to differ more widely from that of plasma than in normal dogs, while thoracic duct lymph may show a relative increase in esterase activity. It may be remarked here that while only one out of eight normal dogs showed flow rates of the liver lymph exceeding

TABLE 1

The distribution of esterase activity between blood plasma and lymph of various sources

DOG	SEX	ESTERASE ACTIVITY mM./L./hour				TOTAL PROTEIN gram %				E/TP			
		P*	LL	CL	TL	P	LL	CL	TL	P	LL	CL	TL
						Normal dogs							
66	M	101	99	29		7.2	6.2	4.8		14	16	6	
67	F	113	120	25	71	4.7	4.6	3.6	3.9	24	26	7	18
21	F	149	102	27		5.5	4.4	2.7		27	23	10	
24	M	88	85	38		5.2	4.7	2.4		17	18	16	
26	M	101	83	19	44	5.3	4.4	2.7	3.4	19	19	7	13
32	M	146	104	62	113	5.8	5.8	3.9	5.4	25	18	16	21
33	M	162	157	51	103	5.2	4.9	4.0	4.3	31	32	13	24
34	F	78	74	26	53	5.6	4.6	3.7	3.8	14	16	7	14
Mean		117	103	35	77	5.6	4.9	3.5	4.3	21	21	10	18
St. D.M.		±11.2	±9.2	±5.2	±13.5	±0.3	±0.3	±0.3	±0.3	±2.3	±2.0	±1.1	±2.1
Mean % of P		100	89	29	61	100.0	90.0	63.0	78.0	100	100	49	81
St. D.M., P=100			±4.9	±3.5	±4.9		±2.6	±4.4	±5.4		±5.1	±7.4	±4.8
						CCl_4 treated dogs							
27	M	116	103	29	87	7.2	6.4	4.1	5.1	16	16	7	17
28	M	117	102	15	68	5.9	4.9	2.5	3.6	20	21	6	19
29	M	133	103	19		6.3	5.1	2.7		21	20	7	
30	F	284	273	43	184	4.7	4.6	3.6	3.9	60	59	12	51
37	F	266	250	66	200	5.6	5.3	3.3	4.8	48	47	20	42
43	M	260	248	32	195	4.3	4.1	2.4	3.3	61	61	13	59
Mean		196	179	34	147	5.7	5.1	3.1	4.1	37	37	11	37
St. D.M.		±33.4	±34.7	±7.6	±2.8	±0.5	±0.3	±0.2	±0.3	±8.6	±8.4	±2.2	±4.4
Mean % of P		100	90	17	70	100.0	90.0	56.0	75.0	100	100	30	94
St. D.M., P=100			3.3	2.4	4.3		2.8	5.2	4.4		1.5	3.4	3.9

* P = blood plasma; LL = liver lymph; CL = cervical lymph; TL = thoracic duct lymph.

0.06 cc. per minute, three out of six carbon tetrachloride treated dogs showed liver lymph flow exceeding this value. The mean value for normal dogs was 0.045, whereas that for carbon tetrachloride treated dogs was 0.091. While the experimental procedure chosen does not appear to warrant accurate quantitative comparison, increased liver lymph flow seemed to accompany the liver injury.

The transfusion of albumin was followed consistently by decreases in the rate of

flow of cervical and thoracic duct lymph, accompanied by increases in the total protein content. Changes in liver lymph flow and protein content were not consistent; there was a significant rise in both variables in the case of dog 34; in one other experiment (dog 26) there was no change in flow and little or no change in the total protein content; in the remaining dogs, liver lymph flow decreased.

TABLE 2

The effect of transfusion of concentrated crystalline albumin on the distribution of esterase in plasma and lymph

DOG	EXPERIMENTAL PROCEDURE		PLASMA			LIVER LYMPH			CERVICAL LYMPH			THORACIC DUCT LYMPH		
			Before transfusion	After transfusion	% of change	Before transfusion	After transfusion	% of change	Before transfusion	After transfusion	% of change	Before transfusion	After transfusion	% of change
66	Bled 100 cc.	E	101	93	−8	99	90	−9	28	36	+28			
	trans. 20 cc.													
	25% albumin	E/TP	14	15	+7	16	17	−6	6	6	0			
67	Bled 140 cc.	E	113	94	−17	120	90	−25	25	23	−8	68	71	+4
	trans. 28 cc.													
	25% albumin	E/TP	24	21	−12	26	19	−27	8	7	−12	17	17	0
26	Bled 100 cc.	E	95	75	−21	93	69	−26	22	26	+18	48	42	−12
	trans. 48 cc.													
	25% albumin	E/TP	19	15	−21	22	17	−22	9	8	−11	14	11	−21
34	Trans. 25 cc.	E	78	60	−23	74	60	−19	18	15	−20	56	62	+9
	25% albumin	E/TP	16	12	−25	16	12	−25	7	5	−29	15	15	0
27*	Trans. 48 cc.	E	116	93	−20	100	84	−16	28	30	+7	85	98	+15
	25% albumin	E/TP	15	14	−12	16	14	−12	9	7	−22	16	18	+12
29*	Trans. 48 cc.	E	130	115	−19	110	95	−14	20	24	+20			
	25% albumin	E/TP	23	20	−13	20	17	−15	7	7	0			
37*	Trans. 40 cc.	E	266	216	−19	254	194	−23	66	73	+15	200	200	0
	25% albumin	E/TP	47	38	−19	48	36	−25	20	20	0	41	39	−5
Average		E			−18			−19			+9			+3
		E/TP			−14			−19			−10			−3

* Carbon tetrachloride treated dogs.

Plasma protein content showed the familiar slight rise followed by return toward normal values.

Plasma esterase activity decreased as a result of dilution following the albumin transfusions (table 2). In the present series of experiments, decreases ranged from 9 to 23 per cent. Simultaneously, the esterase activity of liver lymph showed decreases of a similar magnitude in each case. On the other hand, in

cervical and thoracic duct lymph only inconsistent changes of esterase activity could be observed. The ratios of esterase to total protein (E/TP) decreased less markedly in cervical lymph than in liver lymph and in plasma, and remained virtually unchanged in thoracic duct lymph.

In three dogs mesenteric lymph was collected. The results are shown in table 3. It will be noted that the composition of mesenteric lymph resembles the average composition of thoracic duct lymph obtained from dogs with intact

TABLE 3

Esterase distribution in dogs with cannulae in a mesenteric lymph duct

DOG	ESTERASE ACTIVITY				TOTAL PROTEIN				E/TP			
	P*	CL	TL	ML	P	CL	TL	ML	P	CL	TL	ML
	mM./L./hour				gram %							
42	177	36		87	6.2	2.8		4.6	24	12		18
44	110	42	48	104	6.6	4.2	3.8	5.5	20	8.7	13	18.9
46	116	17	27	55	7.1	3.4	3.1	3.9	17	5.0	8.7	13

* P, CL, TL are same as in table 1. ML = mesenteric lymph.

TABLE 4

Esterase activity of lymphoid tissue

	DOG NO.	mM./kgm./hr.
Lymph nodes (mesenteric)	36	41
	39	38
	40	38
Average		39
Lymphocytes	39	8
	40	19
	41	25
	43	13.8
Average		16.4
Plasma Average (1)		100.9
Liver Average (1)		1260.0

mesenteric lymphatics (table 1). By contrast, the thoracic duct lymph which was obtained from two of these three dogs showed low esterase activities and E/TP ratios.

Lymph nodes and lymphocytes were examined for their esterase content. The results are tabulated in table 4 together with data regarding the average esterase activity of normal dog plasma and liver. The lymphoid tissues contain little esterase.

DISCUSSION. One purpose of these studies was to determine whether the

release of esterase from the liver could be detected by the study of liver lymph. Liver lymph composition can be a useful tool for studies of protein metabolism only if significant qualitative or quantitative differences between the proteins of liver lymph and of plasma can be detected. McCarrell, Thayer and Drinker (7) have found that the A/G ratios of liver lymph and of blood plasma were very close to each other, and that liver lymph contains about 90 per cent of the total protein content of blood plasma. In the course of the present studies these observations have been confirmed with regard to the total protein content and have been strengthened by the observation of similar cholinesterase activities in liver lymph and blood plasma and of identical ratios E/TP. Even when the composition of plasma was altered, either over a two-day period by the administration of carbon tetrachloride, or within 30 to 60 minutes by the injection of esterase-free albumin, the liver lymph reflected the protein composition of the plasma. The similarity of liver lymph to plasma was emphasized by the results of the intravenous administration of the dye T-1824, which is known to be firmly linked to plasma albumin. Here again, within less than 10 minutes the dye appeared in the liver lymph in concentrations slightly below those found simultaneously in blood plasma. These data suggest that passage of lymph from the blood plasma through the liver occurs at a rate which is sufficiently higher than the rates of plasma protein interactions with the hepatic parenchyma to prevent the detection of overall shifts of protein out of or into the hepatic cells.

The second question was the possible production of esterase by lymphocytes. The data presented appear to rule out the possibility that plasma esterase could be formed in the lymphocytes and liberated into the blood and lymph plasma by a process analogous to that involved in the appearance of globulins in the plasma. Even if a low turnover rate of plasma esterase were assumed, the esterase content of the lymphocytes is so low that the rate of lymphocytolysis required to account for the plasma esterase activity would be unreasonably large. This view is supported by preliminary results[5] with methyl bis-β-chlorethyl amine hydrochloride. This agent has been shown to produce massive lymphocytolosis if given intravenously. Yet, so far, no changes in the plasma esterase levels of dogs or of patients so treated have been observed. Present evidence does not rule out the possibility that lymphoid tissue may produce the enzyme and release it into the plasma without actual cell destruction. The very low relative esterase activity of cervical lymph which has passed through a lymph node may be taken to argue against such an assumption.

Several reports have pointed out that non-visceral lymph contains relatively less globulin than does plasma. Thus (8) the mean ratio of globulin to total protein in canine cervical lymph was found to be 82.5 per cent of that of the plasma in the same animals. In calves' leg lymph electrophoretic study (9) showed an average ratio of α-globulin to total protein 76 per cent of that of the serum in the same animal (corresponding figures for β-globulin, 95 per cent; for

[5] The authors are indebted to Dr. Sidney Farber for making available the nitrogen mustard used in these exploratory studies as well as the clinical material. These data were obtained on two dogs and on two patients.

γ-globulin, 85 per cent). The ratio E/TP in cervical lymph, however, is only 49 per cent of this ratio in the blood plasma in normal dogs. Increased cholinesterase activity of blood plasma, brought about by liver injury (carbon tetrachloride), is not accompanied by a significant increase of the esterase activity or the ratio E/TP in cervical lymph. If the bulk of the lymph proteins represents plasma proteins which have passed through the walls of the capillaries, then in the cervical region plasma esterase leaves the blood stream considerably less readily than any one of the electrophoretic components of the globulin complex, or than the total globulin fraction, as obtained by salting out procedures. The differences in the relative rates of penetration of different proteins through the capillary walls, indicated by these results, are considerably larger than had been suspected previously.

In contrast to cervical lymph or liver lymph, each of which comes from a single fairly uniform region, thoracic duct lymph is a mixture of lymph from a number of regions. According to Drinker (10) 95 per cent of thoracic duct lymph is derived from the liver and the small intestine. The above data regarding the composition of mesenteric lymph would be compatible with this assumption if mesenteric lymph supplied the bulk of thoracic duct lymph. On the other hand, thoracic duct lymph in two dogs, the mesenteric lymphatics of which had been cannulated, showed a normal flow rate, but very low esterase and total protein levels. These findings suggest that a considerable amount of thoracic duct lymph in apparently healthy animals can be derived from regions other than the liver and the small intestine.

SUMMARY

1. The cholinesterase activity and the total protein content of samples of blood plasma and of hepatic, cervical, thoracic duct, and mesenteric lymph have been determined in dogs.

2. In normal dogs liver lymph contains about 90 per cent of the esterase activity of an equal volume of plasma and an identical ratio of esterase activity to total protein. These relations persist when plasma esterase has been elevated by treatment of the animals with carbon tetrachloride or reduced by the injection of concentrated albumin solutions.

3. In relation to total protein, the esterase activity of cervical lymph is 49 per cent of that of plasma. The esterase thus appears to pass from plasma to lymph less readily than other globulin components of plasma, which appear in greater proportion in the lymph.

4. Thoracic duct lymph and mesenteric lymph show a composition intermediate between cervical and liver lymph. In two dogs in which the mesenteric trunk was cannulated, lymph with very low esterase and total protein content was obtained from the thoracic duct.

5. Analyses of lymph nodes and of lymphocytes derived from lymph nodes showed low esterase activity. It appears unlikely that these tissues are involved in the production of plasma esterase

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THE USE OF THE SHAY RAT IN STUDYING ANTI-ULCER SUBSTANCES[1]

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Shay and his associates (1) have described a procedure for the production of perforating type lesions in the fore-stomach of the rat in a very short period of time. In its essentials this procedure involves fasting rats for 48 hours followed by ligation of the pylorus under light anesthesia. Gastric secretions collect in the stomach and extensive lesions of the rumen are observed within 8 to 18 hours. For the past two years we have been employing this procedure as a means of assaying such anti-secretory extracts as "enterogastrone" and "urogastrone". Recently, Pauls, Wick and MacKay (2) reported on the satisfactory application of the Shay rat to the assay of urine extracts. On the other hand Morris, Grossman and Ivy (3) have reported that this method is of no value in the assay of the anti-ulcer properties of enterogastrone.

The purpose of this communication is to present further information regarding the use of the Shay rat in the assay of so-called anti-ulcer substances and to attempt an explanation of the mechanism whereby the gastric lesions are suppressed by extracts of intestines and urine.

METHOD. Rats weighing 125–150 grams were fasted for 48 hours. At the end of this time they were lightly anesthetized with ether and through a short mid-line incision the pylorus was ligated. When such factors as age and weight of the rats, length of fasting period and absence of excess surgical trauma were controlled, hemorrhagic lesions occurred in the fore-stomach of all rats within 16 hours. In our hands a 16-hour assay period was necessary in order to obtain severe ulceration in all animals. However, it must be stressed that others who have reported their observations on the Shay rat have obtained consistent ulceration in as short a period of time as 8 hours. By means of check assays we have shown that there were no qualitative differences between our 16 hour assay and an 8 hour assay employed by another laboratory, but as a consequence of the longer assay period larger doses of substances to be assayed were required.

When a substance was to be assayed for its anti-ulcer activity it was administered parenterally, usually intraperitoneally, at the time of operation. In order to grade the activity of the anti-ulcer substances a system of scoring the severity of ulceration was designed. Animals showing the most severe ulceration in the 16-hour assay period were scored +4. Decreasingly severe ulceration was scored +3, +2, +1, and finally 0 for those animals completely free of any gross appearance of gastric lesions. The average of the ulcer scores in any group of animals was multiplied by 100 to give the ulcer index.

[1] A preliminary report of these data was presented at the meetings of the Federation of American Societies for Experimental Biology, Chicago 1947.

RESULTS. Table 1 illustrates the constancy of ulceration in a rather large series of rats. The mean ulcer index for all groups is 330 and it will be seen that the deviation from the mean is relatively small. The total volume of gastric contents at the end of the 16-hour assay, total and free acid concentration and pepsin concentration as measured by the Bucher hemoglobin method (4) are given also. As a consequence of sloughing tissue, hemorrhage and the establishment of a high intragastric pressure the volume and acid concentration at the end of the 16-hour assay does not give an accurate indication of gastric secretion at shorter time intervals when the gastric lesions were being formed.

The inhibition of ulceration by extracts of intestines and urine is shown in table 2. The acid extract of intestines was prepared by leaching fresh hog intestines (upper 6 ft.) with 0.4 per cent concentrated hydrochloric acid. The extract was filtered and lyophilized. Enterogastrone was prepared from fresh

TABLE 1

Ulcer index and gastric juice composition of Shay rats 16 hours after operation

GROUP NO.	NO. RATS	ULCER INDEX	VOLUME	TOTAL ACID	FREE ACID	PEPSIN
			ml.	*units/100 cc.*	*units/100 cc.*	*units/100 cc.*
1	10	340	11.8	30	12	
2	7	330	10.1	55	45	
3	5	320	11.2	77	41	
4	8	380	7.2	46	17	
5	7	270	10.6	57	53	4.36
6	12	360	7.9	50	41	3.28
7	12	300	11.6	66	57	5.31
8	12	320	8.4	52	44	3.20
9	8	330	10.5	64	48	3.48
10	10	320	9.7	38	25	3.59
11	8	380	10.6	50	33	
Total	99					
Average		330	10.0	53	38	3.87

hog intestines by the picric acid method of Greengard *et al.* (5). Urine extract (Wick) was prepared from fresh human male urine by the charcoal adsorption method of Wick *et al.* (6) and urogastrone by a procedure similar to that of Gray *et al.* (7). The extracts are listed in order of increasing activity. We have made several crude acid extracts of hog intestines that have given the same general order of activity as the preparations illustrated here. However, many extracts of this type have shown little activity or a complete absence of activity at the dosage levels presented here. This same variation in the activity of different preparations of enterogastrone has been observed in our laboratory. Another point to be stressed is the rather large dosage of some of the extracts required in order to obtain a significant depression in gastric ulceration. With the exception of urogastrone most preparations had to be administered in doses of 50 mgm. or more per rat in order to obtain definite lowering of the ulcer index.

Crude acid extracts of intestines, the salt cake employed in making enterogastrone, and urogastrone have been administered orally to rats under a variety of conditions. In no case was the orally administered material effective in reducing the ulcer index.

TABLE 2

Anti-ulcer activity of extracts of urine and intestines in the 16 hour assay

GROUP NO.	TYPE PREPARATION	NO. RATS	DOSE	ULCER INDEX
			mgm./rat	
1	Controls	99	0	330
6	Acid extract of intestines	17	200	71
		11	100	145
2		6	50	270
3	"Enterogastrone"	11	50	184
4	Urine extract (Wick)	10	50	158
7	"Urogastrone"	15	50	20
5		9	25	130

TABLE 3

Inhibition of gastric secretion by extracts of urine and intestines in the 5 hour assay

GROUP NO.	TYPE PREPARATION	NO. RATS	DOSE	VOLUME	TOTAL ACID	FREE ACID	PEPSIN
			mgm./rat	*ml.*	*units/100 cc.*	*units/100 cc.*	*units/100 cc.*
1	Control	23	0	6.3	107	101	4.15
6	Acid extract of	10	200	0.77	60	32	5.40
2	intestines	10	50	2.68	91	82	4.18
3	"Enterogastrone"	10	100	0.95	48	28	7.90
		10	50	1.67	61	48	5.05
4	Urine extract (Wick)	10	50	1.57	43	30	4.50
7	"Urogastrone"	10	50	0.40	7	0	1.92
5		10	25	0.90	20	3	3.90
		10	5	1.85	71	47	4.50

Since the analysis of gastric secretion at the end of the 16-hour assay period could not be used for an interpretation of the anti-secretory activity of the various extracts, we have utilized the Shay rat in a 5-hour gastric secretion assay. The rats were prepared as previously described. The substance to be tested was administered intraperitoneally at the time of operation and at the end of a 5-hour period they were killed and the entire stomach contents taken for analysis. Table 3 shows the results of this 5-hour assay. In this short period of time no

evidence of ulceration was observed. It will be seen that with each of the extracts that had been previously shown to have anti-ulcer activity there was a reduction in the volume of gastric contents and the concentration of total and free acid. In one case only was there a significant reduction in the pepsin concentration and this was with the largest dose of urogastrone that was employed. Peculiarly enough, the intestinal extracts appeared to cause an elevation of the pepsin concentration.

It was part of the purpose of this study to attempt a correlation between the anti-secretory and the anti-ulcer effects of these extracts. In figure 1B the volume of gastric contents at the end of the 5-hour assay has been plotted

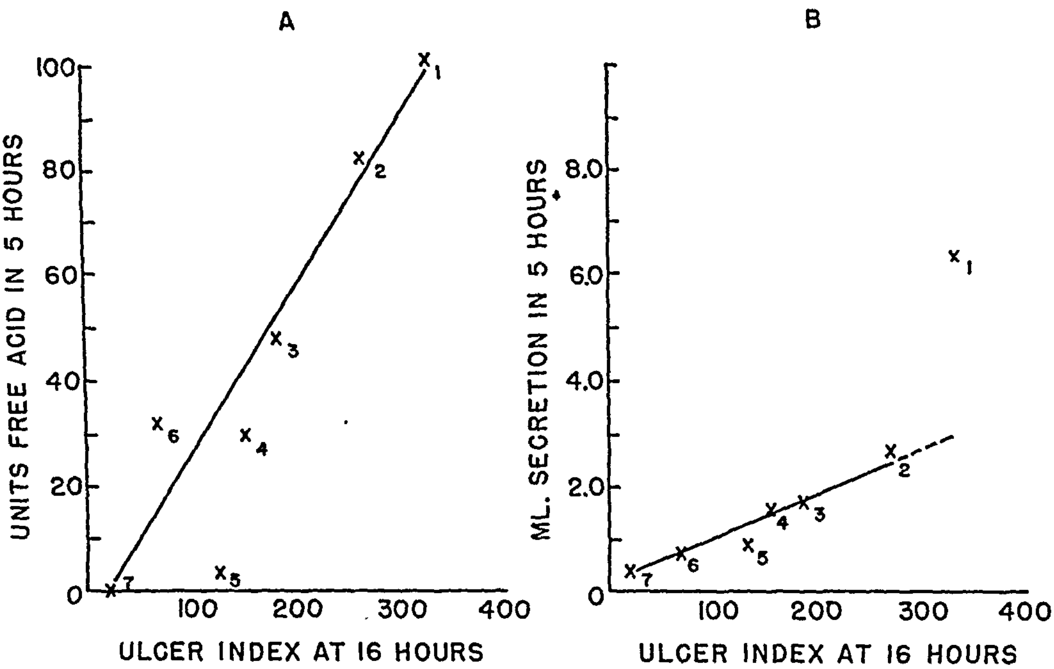

Fig. 1A. Relationships between concentration of HCl in gastric juice collected at the end of 5 hour assay periods and the ulcer index as measured in 16 hour assays.

Fig. 1B. Relationships between volume of gastric juice secreted during the 5 hour assay periods and ulcer index as measured in 16 hour assays.

The numbers beside each of the points correspond to the group numbers as given in tables 1 and 2.

against anti-ulcer activity as determined in the 16-hour assay. For each point the same preparation at the same dosage level was employed for both anti-secretory and anti-ulcer determinations. It will be seen that in general the depression in gastric secretion parallels inhibition of ulceration. It would appear that an initial depression in secretion is manifested before inhibition of ulceration becomes evident.

In figure 1A the concentration of free acid in the 5-hour assay period is plotted against the ulcer index in the 16-hour assay. Again a general relationship seems to hold between inhibition of ulceration and secretion.

Although a relationship is observed between inhibition of ulceration and both volume of gastric juice and concentration of acid in secreted gastric juice it

is most interesting to note that no such relationship was observed for pepsin concentration. On several occasions it has been observed that with exceptionally potent dosages of an anti-ulcer substance which incidentally caused a very marked depression in volume secreted, there was some depression in pepsin concentration. It was observed that with the higher doses of urogastrone, the pH of the gastric contents was neutral and the change in pepsin concentration could be due to irreversible inactivation of the pepsin present in the gastric juice. With smaller doses of the substances that caused a significant inhibition of ulceration no conclusive change in pepsin concentration was observed.

Discussion. Shay and his collaborators (1) have suggested that the formation of lesions in the rumen is due largely to the fact that this region of the stomach not normally exposed to digestive concentrations of acid and pepsin is unable under the condition of the experiment to cope with the secretions that come in contact with it. By means of a stomach tube we have introduced into the stomach of the Shay rat 4 cc. of an acid-pepsin mixture roughly corresponding in concentration to the natural gastric juice of the rat, but have not observed lesions after periods up to 5 hours. Of course this volume of liquid engorges the stomach to such an extent that all parts are in contact with the digestive mixture. On the other hand, if 8 or 10 cc. of the same artificial gastric juice is introduced in the stomach very severe ulceration may occur within an hour. Since the concentration of acid and pepsin is the same in the two cases it has been concluded that the reason for the severe ulceration in the second case is the suddenly developed high intragastric pressure. This large volume causes a marked distention of the stomach and possibly acts by increasing the penetration of the artificial gastric juice into the mucosal tissue or causes localized ischemia by occlusion of the vascular bed. The importance of intragastric pressure should not be minimized in formulating any hypothesis of the mechanism whereby gastric lesions appear in the Shay rat.

When ulceration is inhibited by extracts of intestines or urine there is a decrease in the volume of gastric juice as well as a decrease in the concentration of acid in the secretions. A rough parallelism exists between the degree of ulcer inhibition and the depression in volume of acid concentration. Therefore, it would appear that in the Shay rat inhibition of gastric lesions by these extracts can be accounted for solely on the basis of their anti-secretory properties.

SUMMARY

The Shay rat is of value in the assay of the anti-secretory properties of parenterally administered extracts of urine and intestines. By this procedure several different extracts have been assayed and their general order of activity appeared to agree with results obtained by other procedures that have been quoted in the literature. None of these preparations was active when given orally. It has been concluded that these extracts inhibit ulceration in the Shay rat by decreasing the volume of gastric secretion and the concentration of acid. They do not seem to have a specific effect upon pepsin secretion. No evidence has been found that an anti-ulcer activity other than that of secretory inhibition plays a part in the prevention of ulcers in this preparation.

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EFFECT OF LEVEL OF THYROID ACTIVITY ON RESPONSE OF OVARIECTOMIZED RATS TO ESTRONE[1]

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That the secretion of the thyroid gland affects the response of the vaginal mucosa of the castrate rat to estrogens was first suggested by Reiss and Pereny (1928). They reported that the thyroid hormone was an antagonist of the ovarian hormone as evidenced by the fact that subcutaneous injections of thyroid autocoid not only prevented heat in normal female rats and mice but also inhibited the action of the ovarian hormone in castrate animals.

Their observations were confirmed by Van Horn (1933). He fed desiccated thyroid powder to ovariectomized rats and observed their response to estrone by observing the cellular composition of vaginal smears. He found that approximately three times as much estrogen was required to produce positive estrous smears as was necessary before thyroid feeling.

Halpern and Hendryson (1935) reported that dinitrophenol, though producing an increased metabolic rate, did not completely inhibit estrus in the normal rat. The diestrual phase of the estrous cycle was lengthened considerably, however.

Meyer and Wertz (1938) used desiccated thyroid powder, thyroid globulin and dl-thyroxin and found that treatment with these materials at moderate doses for three days did not interfere markedly with the action of estrone; however, treatment for more than five days increased the estrone threshold.

Observations of the effects of hypothyroidism on the response of ovariectomized rats to estrogens are rather limited. Müller (1938) reported that thyroidectomy was without effect on the response of castrates to estradiol benzoate. This result seems to be contrary to what one would expect. Hypothyroidism might be expected to have an opposite effect to hyperthyroidism.

Richter (1933), on the other hand, reported that vaginal smears taken on thyroidectomized animals with intact ovaries showed a persistence of cornified cells in the vaginal smears at all times as if there was a tendency toward a prolonged estrual phase. At autopsy, the animals also showed hypertrophied uteri.

The lack of quantitative data in the foregoing investigations seems to justify a detailed study of the effects of both hyper- and hypothyroidism on the response of spayed rats to estrone.

METHODS AND RESULTS. *Effects of daily doses of dl-thyroxin*[3]. A series of experiments was conducted to study the effects of both size and duration of thyroxin dosage on the response of ovariectomized rats to estrone.

[1] This paper is part of a thesis submitted to the University of Colorado by W. Langham in partial fulfillment for the requirements of the Ph.D. degree.

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[3] The dl-thyroxin used in these experiments was a synthetic crystalline product purchased from Hoffman-La Roche, Inc., Nutley, New Jersey.

Two hundred castrate rats were used for the experiments. The colony was maintained on a balanced composite diet and their average weight was about 225 grams. The entire colony was injected with 1.5 μg. of estrone. The animals were then divided into 4 groups of fifty animals each. The groups were selected to give approximately equal responses to the estrone dosage.

One group served as the control and received no thyroxin prior to injection of estrogen. The other three groups received different doses of thyroxin daily over various periods of time. On the last day of each period of thyroxin dosage 1.5 μg. of estrone in 0.5 cc. of olive oil was administered by subcutaneous injection. Forty-eight hours later, corresponding to the time of maximum thyroxin effect (Meyer and Wertz, 1939), the response to estrone was determined by the method of "vaginal smears" (Allen and Doisy, 1923).

The effect of doses of 1, 2 and 3 μg. of thyroxin per gram of body weight on the estrous response of ovariectomized rats was studied when the above doses were administered daily for periods of 3, 6, and 10 days. According to Meyer and Wertz (1939), doses of 1, 2, and 3 μg. of thyroxin per gram of body weight administered daily for three days to normal rats produced an increase in oxygen consumption of 13, 20 and 25 to 40 per cent respectively. The thyroxin doses administered in these experiments were based on the initial body weight of the individual animals at the time each series of injections was begun. No change in the dosage was made to correct for weight loss occurring during a series of injections. The thyroxin was dissolved in 0.01 normal sodium hydroxide and injected subcutaneously.

Between successive periods of injection the animals were allowed to recover their lost weight. The recovery of their initial body weight was used as an indication of recovery from thyroxin effect and marked the beginning of the next series of injections. This proved, however, to be a poor indicator and evidence of cumulative effects of successive doses occurred in the results.

A detailed analysis of the effects of size and duration of thyroxin dosage on the weight of these animals is given by plotting percentage loss in body weight against dosage of thyroxin in μg. (fig. 1a) and against days of dosage (fig. 1b). These graphs show that weight change was a logarithmic function of both size and duration of dosage with thyroxin. The failure of the points representing the 2 and 3 μg. doses (fig. 1b) to fall on the uniform curves is probably indication of a cumulative effect as a result of failure to allow a sufficiently long recovery period between series of injections. The points of the curve representing the 1 μg. dose are in better agreement. Animals that received daily doses of 1, 2 and 3μg. of thyroxin per gram body weight for ten consecutive days lost 6.8, 8.4 and 8.8 per cent of their original body weight respectively. As a result of a six day period of injection of the above doses the respective weight losses were 5.9, 7.8 and 8.3 per cent of the original weight. When the dosage period was three days, the weight losses were 3.4, 5.0 and 5.1 per cent respectively.

The data showing the effect of size and duration of thyroxin dosage on the response of ovariectomized rats to 1.5 μg. of estrone are expressed graphically (fig. 2) by plotting dosage of dl-thyroxin in μg. against per cent of animals giving

an estrous response. Reasonably uniform logarithmic curves were obtained for each period of dosage. The curves obtained when days of dosage are plotted against per cent response are not uniformly logarithmic (fig. 3). This is probably a result of failure to allow a sufficiently long recovery period between periods of injection. The groups of animals that received daily doses of 1, 2 and 3 μg. of thyroxin per gram body weight for ten consecutive days were respectively 30, 15 and 12 per cent as responsive to 1.5 μg of estrone as was the control group. As a result of a six day period of injection of the above doses of thyroxin the observed estrous responses were respectively 47, 38 and 31 per cent of that of the controls. When the dosage period was three days the animals in each dosage group were respectively 77, 71 and 68 per cent as sensitive as the controls.

Meyer and Wertz (1939) in their study of the calorigenic efficiency of thyroid material found that in both normal and thyroidectomized rats the influence of thyroxin adminstration on oxygen consumption was a logarithmic function of the dosage. When the foregoing results are considered in connection with their report, it seems reasonably well established that both weight loss and decrease in sensitivity of ovariectomized rats to estrone as a result of thyroxin dosage are directly proportional to the basal metabolic rate. These conclusions are in accord with the hypothesis of Van Horn (1933) that the decrease in sensitivity of hyperthyroid rats to estrone is probably a result of the effects of the increased metabolism on the rate of deactivation and elimination of estrogen.

Some indication of the duration of thyroxin effect was obtained by testing the sensitivity of each group of rats to 1.5 μg. of estrone at 14 and 28 days after the 10-day period of thyroxin injection. These results are shown in table 1. It can be seen from these data that the sensitivity of each group was significantly below that of the control group after 14 days of recovery; after 28 days their response was essentially the same as the controls.

Effect of thyroparathyroidectomy. One hundred and ten Yale strain rats were ovariectomized when about 60 days of age. Four weeks later they were injected with 1.3 μg. of estrone and divided into two groups giving approximately equal response to the estrone dosage. One group was thyroparathyroidectomized immediately. The other group was kept as a control.

No attempt was made to avoid removing the parathyroids during the thyroid operation. Forty-eight hours after operation over 50 per cent of the animals showed signs of parathyroid deficiency. Those showing severe symptoms were injected immediately with calcium gluconate. The regular diet was then supplemented daily with fresh skim milk to which was added a quantity of calcium lactate. The controls were treated likewise. This treatment was carried on throughout the experiment. No more symptoms of parathyroid tetany were observed.

At the end of the experiment the animals were autopsied and the tracheal region examined for the presence of thyroid tissue. Five of the fifty-five animals showed incomplete thyroidectomy; these were eliminated from the final results.

Beginning 10 days after thyroparathyroidectomy the response of each group to a subcutaneous injection of 1.3 μg. of estrone in olive oil solution was deter-

mined at 14-day intervals. During the fifth interval the thyroidectomized colony received three daily injections of 0.1 μg. of thyroxin per gram of body weight, the last thyroxin administration coming on the same day as the estrone injection. The response to estrone was determined 48 hours after injection in the usual way. The data collected are presented graphically in figure 4.

These results show that thyroparathyroidectomy produces an appreciable increase in the sensitivity of ovariectomized rats to estrone. The maximum

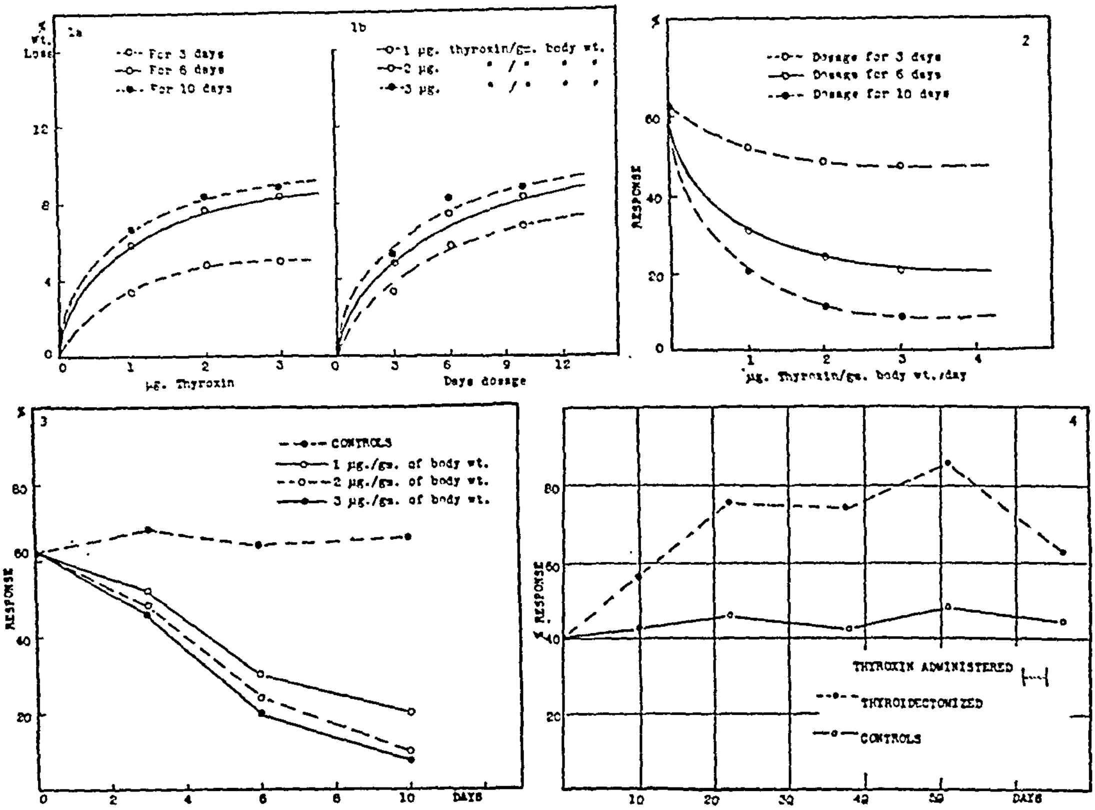


Fig. 1. Relation of thyroxin administration to weight loss by ovariectomized rats. 1a. Per cent weight loss versus size of dose. 1b. Per cent weight loss versus days of dosage.

Fig. 2. Effect of size of thyroxin dosage on response of ovariectomized rats to 1.5 μg. of estrone.

Fig. 3. Effect of days of thyroxin dosage on response of ovariectomized rats to 1.5 μg. of estrone.

Fig. 4. Effect of thyroparathyroidectomy on response of ovariectomized rats to 1.3 μg. of estrone.

effect was produced about 38 days after operation. Thirty-eight days after operation the thyroparathyroidectomized group was 76 per cent more sensitive to 1.3 μg. of estrone than was the control group. That the increase in sensitivity to estrogen was, at least in part, due to the hypothyroid condition of the animals was shown by the fact that daily administration of 0.1 μg. of thyroxin per gram of body weight for 3 days resulted in a decrease in sensitivity to only 41 per cent above the controls. The choice of thyroxin dosage was based on the report of

Meyer and Wertz (1939) that thyroidectomized rats were 25 to 30 times more sensitive to thyroxin than normal rats. Although the above dosage did not completely lower the response to normal it produced a significant effect.

The increase in sensitivity of ovariectomized rats to estrone as a result of thyroidectomy is in keeping with Van Horn's (1933) hypothesis that the level of metabolism affects the rate of elimination of estrogens from the body, thereby affecting response to estrogen administration.

Effect of thiourea. An obvious way of studying the effect of hypothyroidism on response of ovariectomized rats to estrone is to inhibit the thyroid with some of the goitrogenic agents studied by MacKenzie and MacKenzie (1943) and Astwood et al. (1943).

Virgin female rats were spayed at 50 to 60 days of age. Fifteen days after operation they were injected with 1.3 μg. of estrone and divided into four groups of 45 animals each. The four groups were chosen to give approximately equal response to the estrone injection.

TABLE 1

The recovery of sensitivity to 1.5 μg. of estrone after 10-day injection period with various doses of thyroxin

DOSE THYROXIN μg./gm. BODY WT.	DAYS RECOVERY AND % RESPONSE		
	0	14	28
0	66	68	66
1	20	52	68
2	10	38	74
3	8	34	70

One group was kept as a control while the others received 0.05, 0.1 and 0.3 per cent of thiourea respectively in the drinking water.

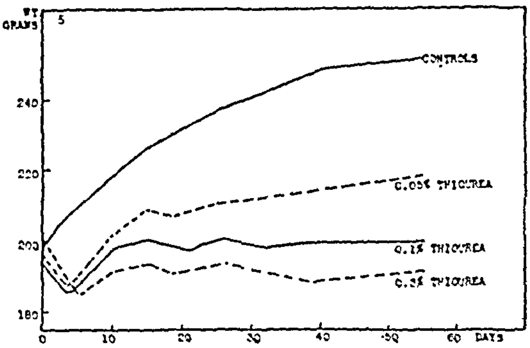
During the first 24 hours 15 of the 135 animals (11 per cent) receiving the thiourea died. Eleven of the 15 were in the group receiving the lowest dosage (0.05 per cent). Autopsy revealed that the lungs were covered with hemorrhagic areas and that the chest cavity was almost completely filled with a clear fluid.

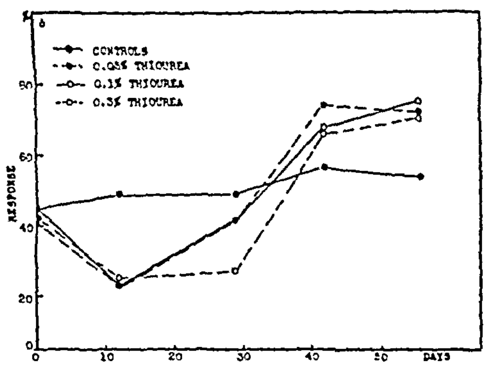
Throughout the experimental period records were kept of the weights of the animals in each group. These data are presented graphically in figure 5. The toxicity of the initial dose of thiourea was evidenced by marked loss in weight during the first 24 hours.

Beginning 12 days after the start of thiourea treatment the response of each group to 1.3 μg. of estrone was determined at approximately fourteen-day intervals until 4 determinations were made.

The data showing the effects of the various doses of thiourea on the response of ovariectomized rats to estrone are presented in figure 6 by plotting the percentage of animals giving an estrus response versus days of thiourea dosage. These results were rather surprising in that a significant *decrease* in sensitivity

occurred at first, after which the sensitivity began to increase. After 12 days of thiourea treatment those animals that received 0.05 and 0.1 per cent thiourea gave a response to 1.3 μg. of estrone which was only 46 per cent of that of the normals. After 42 days of treatment the groups receiving 0.05 and 0.1 per cent thiourea attained a degree of sensitivity approaching that of surgically thyroidectomized animals. The immediate decrease in sensitivity to estrone may have been a manifestation of the toxic symptoms of the initial dose of thiourea. After the initial toxic symptoms were overcome the effects of hypothyroidism may have become predominant resulting in the expected increase in sensitivity.





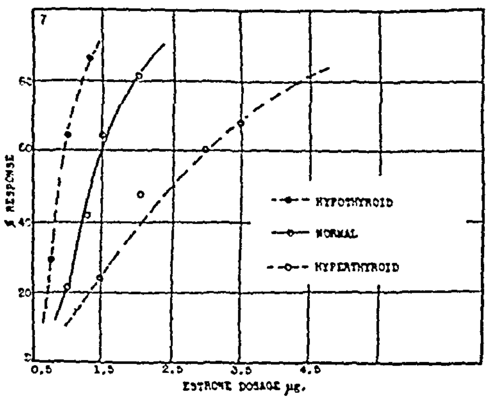


Fig. 5. Effect of thiourea on weight gain of ovariectomized rats.
Fig. 6. Effect of thiourea on response of ovariectomized rats to 1.3 μg. of estrone.
Fig. 7. Dosage response curves of ovariectomized rats to estrone at three different levels of thyroid function.

The extreme toxic nature of thiourea may also explain why the group of animals receiving 0.3 per cent thiourea remained somewhat less sensitive to estrogen than did the other two groups.

To make certain that inhibition of the thyroid gland had resulted from the thiourea treatment 6 animals from each group were killed at the end of the experiment and the fresh weights of the thyroids determined. The data are given in table 2. These data show that extreme hyperplasia of the thyroid had occurred. In addition to being greatly enlarged, the thyroid glands in the treated rats were strikingly hyperemic.

Effect of level of thyroid function on the rat unit of estrone. In the previous

experiments the work was so arranged that an additional injection provided data from which the rat unit of estrone could be determined on groups of rats at three different levels of thyroid function. The unit was determined on the thyroparathyroidectomized colony, a control colony and a group that had received 2 μg. of thyroxin per gram of body weight for 6 days prior to each estrone injection. The dosage response curve for each colony is given in figure 7.

These results show that the level of thyroid function significantly affects the rat unit of estrone For the normal castrate colony the rat unit was 1.33 μg. as compared to 2.5 for the hyperthyroid group and 0.86 for the thyroparathyroidectomized colony.

TABLE 2

Effect of thiourea on fresh weight of the thyroid gland of ovariectomized rats

DOSAGE GROUP	AV. RAT WT.* GM.	AV. GLAND WT* MG.	AV. GLAND WT.* MGM./100 GM. BODY WT.
Control	255	11.5	4.5
0.05%	228	21.8	9.5
0.1%	195	21.7	11.1
0.3%	168	23.6	14.0

* Av. of 6 animals.

SUMMARY

The daily administration of dl-thyroxin by subcutaneous injection at dosage levels of 1, 2 and 3 μg. per gram of body weight for 3, 6 and 10 days decreased the estrus response of ovariectomized rats to 1.5 μg. of estrone as determined by the method of vaginal smears. Both the weight loss and the decrease in sensitivity to estrone appeared to be logarithmic functions of the thyroxin dosage. Twenty-eight days after a 10-day period of thyroxin dosage the response to estrone had returned to normal.

Thyroparathyroidectomy resulted in an increase in estrus response of ovariectomized rats to 1.3 μg. of estrone. The response of a group of 50 operated animals was 76 per cent above that of the controls 38 days after operation. The increased sensitivity was partially corrected by three daily injections of 0.1 μg. of thyroxin per gram of body weight.

The daily administration of thiourea at levels of 0.05, 0.1 and 0.3 per cent in the drinking water of ovariectomized rats over a period of 70 days inhibited growth and produced marked hyperemia and enlargement of the thyroid gland. The estrus response of spayed rats to 1.3 μg. of estrone was first reduced and then increased as dosage with thiourea continued. After 42 days of dosage the response to estrone approached that of surgically thyroparathyroidectomized rats.

The rat unit of estrone was determined at three different levels of thyroid function. The unit as determined on a normal ovariectomized colony was 1.33 μg. On a thyroparathyroidectomized colony it was 0.86 μg., and when determined on a colony rendered hyperthyroid by 6 daily injections of 2 μg. of thyroxin per gram of body weight the unit was 2.50 μg.

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INDEX

ABSORPTION of chloride from small intestine, carotid sinus and, 149.
—— of saline, intestinal, blood pressure and, 466.
Acclimatization to extreme cold, 99.
Acetylcholine in determination of circulation time, 504.
Action potentials in DCA treated rats, 451.
ADAMS, A. D., JR. See BRUNICH, MCWILLIAMS, MASON, ADAMS and ERSHOFF, 551.
Adrenal cortex after hypophysial stalk extirpation, 222.
—— —— extract, effect of, on blood glucose level, 423.
—— —— ——, —— ——, on liver and kidney, 580.
—— response to total body x-radiation, 480.
Adrenalin, action of, on spinal neurones, 37.
——, central hyperglycemic action of, 588.
——, effect of, on blood sugar and hypoxia, 321.
—— injection, effect of, on blood flow, 181.
Adrenocorticotropic hormone, effect of, on urinary glucose and nitrogen in diabetes, 400.
ALBANESE, A. A., V. IRBY, J. E. FRANKSTON and M. LEIN. The effect of carbohydrate feeding on the output of urinary amino acid, 389.
ALEXANDER, I. E. See SIEGEL, ALEXANDER and STUCKEY, 729.
ALEXANDER, R. S. and E. A. WEBB. An analysis of changes in the contour of the femoral arterial pulse during hemorrhagic shock, 272.
Allantoin, blood uric acid and, after nephrectomy and hepatectomy, 677.
Alloxan, effect of, on muscle glycolysis, 613.
Altitude, effect of oxygen, exercise and, on breath-holding time, 142.
Alveolar air during simulated flights to high altitudes, 202.
Amino acid, urinary, effect of carbohydrate feeding on output of, 389.
Amino acids, blood, effect of insulin on, 682.
ANNEGERS, J. H. AND A. C. IVY. The effect of dietary fat upon gastric evacuation in normal subjects, 461.
Anoxia, acute, effect of, on pulmonary artery pressure, 315.
——, anoxemic, effects of, on heart muscle, 493.
——, discontinuous chronic, effect of, on liver glycogen stores, 65.
—— from exposure to low barometric pressure, 1.
ARANA, R. See LEIMDORFER, ARANA and HACK, 588.
Arterial pulse, femoral, in hemorrhagic shock, 272.
Arteries of cat, streamline blood flow in, 52.
Artery pressure, pulmonary, effect of acute anoxia on, 315.

BALDWIN, J. See COURNAND, MOTLEY, HIMMELSTEIN, DRESDALE and BALDWIN, 267.
BALL, Z. B., R. H. BARNES and M. B. VISSCHER. The effects of dietary caloric restriction on maturity and senescence, with particular reference to fertility and longevity, 511.
Barbiturates, effect of, on fatigue produced by prolonged wakefulness, 253.
BARNES, R. H. See BALL, BARNES and VISSCHER, 511.
——. See RISLEY, RAYMOND and BARNES, 754.
BENNETT, L. L. and C. H. LI. The effects of the pituitary growth and adrenocorticotropic hormones on the urinary glucose and nitrogen of diabetic rats, 400.
Bile, recovery of intravenously injected BSP from, 299.
Bioassay of steroid hormones, 444.
BISCHOFF, F. and H. R. PILHORN. Bioassay of steroid hormones using aqueous sodium lauryl sulfate solution as the dispersing agent, 444.
Blood,-acetylcholine in determination of circulation time, 504.
—— amino acids, effect of insulin on, 682.
——, bone-marrow, O_2 in, and erythropoiesis, 618.
——-borne vasotropic substances in shock, 239.
—— coagulation factor, fibrinogen and, in stored plasma, 405.
—— ——, factors in, 409.
—— flow and activity, temperature, in skeletal muscle, 705.

Blood flow and blood content of skin, 122.
—— ——, effect of adrenalin injection on, 181.
—— —— in arteries of cat, 52.
—— ——, peripheral, control of, 304.
—— glucose level, effect of adrenal cortex extract on, 423.
——, oxygen content of, at low barometric pressures, 1.
—— plasma, esterase activity of, 746.
—— —— protein, physiological effects of, 471.
—— ——, recovery of a pressor principle from, 353.
—— ——, specific gravity of, in water privation, 729.
—— ——, stored, fibrinogen and coagulation factor in, 405.
—— —— volume and thiocyanate space in famine edema, 170.
—— pressure and intestinal absorption of saline, 466.
—— —— flow studies on renal circulation, 534.
—— ——, pulmonary artery, 315.
—— —— tracings from left auricle and pulmonary veins, 267.
——, renal venous, renin content of, 198.
—— sugar and hypoxia, effect of adrenalin on, 321.
—— ——, body temperature and, in the chicken, 67.
—— —— levels after ingestion of sucrose, 263.
—— uric acid and allantoin after nephrectomy and hepatectomy, 677.
—— volume, effect of temperature and posture on, 628.
BOBB, J. R. R. and H. D. GREEN. Effect of heparin on ischemic compression shock, 697.
—— —— ——. Rôle of the kidney in resistance to ischemic compression shock, 700.
BODNAR, S. R. See GUEST, MURPHY, BODNAR, WARE and SEEGERS, 471.
Body temperature and blood sugar in the chicken, 67
Bone marrow blood, O_2 in, and erythropoiesis, 618.
BORISON, H. L. See WANG and BORISON, 712, 722.
Boron, effects of adding, to potassium-deficient diet, 520.
Brain in convulsions, changes in, 27.
BRAUER, R. W. and E. HARDENBERGH. Distribution of esterase in lymph from from various regions and in relation to lymphoid tissue, 746.
Breath-holding time, 142.
BRECKENRIDGE, C. G. See KELLER and BRECKENRIDGE, 222.
BRITTON, S. W., V. A. PERTZOFF, C. R. FRENCH and R. F. KLINE. Circulatory and cerebral changes and protective aids during exposure to acceleratory forces, 7.
Bromsulphalein, intravenously injected, removal of, by liver, 299.
BRUNISH, V. H., H. B. MCWILLIAMS, G. D. MASON, A. D. ADAMS, JR. and B. H. ERSHOFF. Effects of vitamin imbalance under conditions of ad libitum feeding and reduced caloric intake, 551.
BUCHER, G. R. and A. C. IVY. Disappearance of uropepsin from the urine of gastrectomized cats, 415.
Burn shock, efficacy of infusion fluids in, 428.
BYER, J. See HARPUDER, BYER and STEIN, 181.
BYERS, S. O., M. FRIEDMAN and M. M. GARFIELD. The blood uric acid and allantoin of the rat after nephrectomy and hepatectomy, 677.

CALORIC intake, reduced, vitamin imbalance in, 551.
—— restriction, effects of, on fertility and longevity, 511.
Carbohydrate feeding, effect of, on output of urinary amino acid, 389.
Cardiac. *See* Heart.
Cardiovascular reflex mechanism, carotid sinus, 712, 722.
Carotid-mandibular reflex in gasping, 358.
Carotid sinus and absorption of chloride from small intestine, 149.
—— —— cardiovascular reflex mechanism 712, 722.
Cerebral changes, circulatory and, from acceleratory forces, 7.
CHAMBERS, R. and B. W. ZWEIFACH. Blood-borne vasotropic substances in experimental shock, 239.
Chloride, absorption of, from small intestine, carotid sinus and, 149.

CHRISTENSEN, W. R. See FERRIS, FORSTER, PILLION and CHRISTENSEN, 304.
Circulation, renal, pressure flow studies on, 534.
—— time, acetylcholine in determination of, 504.
Circulatory and cerebral changes from acceleratory forces, 7.
CISNEROS, F. See WILBURNE, SCHLICHTER, GROSSMAN and CISNEROS, 504.
COHN, C., R. LEVINE and D. STREICHER. The rate of removal of intravenously injected bromsulphalein by the liver and extra-hepatic tissues of the dog, 299.
Cold, extreme, acclimatization to, 99.
COLFER, H. F. and H. E. ESSEX. The distribution of total electrolyte, potassium and sodium in the cerebral cortex in relation to experimental convulsions, 27.
Convulsions, changes in brain in, 27.
COREY, E. L. An experimental study of explosive decompression injury, 607.
COURNAND, A., H. L. MOTLEY, A. HIMMELSTEIN, D. DRESDALE and J. BALDWIN. Recording of blood pressure from the left auricle and the pulmonary veins in human subjects with interauricular septal defect, 267.
——. See MOTLEY, COURNAND, WERKO, HIMMELSTEIN and DRESDALE, 315.

DANLEY, K. S. See SHIPLEY and DANLEY, 84.
DCA treated rats, action potentials in, 451.
Decompression injury, explosive, 607.
—— sickness on re-ascent to high altitudes, 133.
DEMPSTER, W. T. and J. C. FINERTY. Relative activity of wrist moving muscles in static support of the wrist joint; an electromyographic study, 596.
DENISON, A. B. See LIPTON, DENISON and GREEN, 693.
DENSLOW, J. S., I. M. KORR and A. D. KREMS. Quantitative studies of chronic facilitation in human motoneuron pools, 229.
Depancreatized herbivora, action of insulin in, 46.
DEXTER, L. See HAYNES and DEXTER, 190.
——. See HAYNES, DEXTER and SEIBEL, 198.
Diabetes, effect of hormones on urinary glucose and nitrogen in, 400.
——, pituitary and ovarian disfynction in, 84.
Dietary fat, effect of, on gastric evacuation, 461.
DONHOFFER, S. and J. VONOTZKY. The effect of environmental temperature on food selection, 329.
—— ——. The effect of thyroxine on food intake and selection, 334.
DRESDALE, D. See COURNAND, MOTLEY, HIMMELSTEIN, DRESDALE and BALDWIN, 267.
——. See MOTLEY, COURNAND, WERKO, HIMMELSTEIN and DRESDALE, 315.

EDEMA, famine, plasma volume and thiocyanate space in, 170.
Electrolyte, Na and K changes in brain in convulsions, 27.
Electromyographic studies on sciatic nerve, 558.
ELLIOTT, H. W. See RALSTON, TAYLOR and ELLIOTT, 52.
Endocrine glands, urogastrone excretion after extirpation of, 373.
Energy-rich phosphate supply of failing heart, 733.
ERSHOFF, B. H. See BRUNISH, McWILLIAMS, MASON, ADAMS and ERSHOFF, 551.
Erythropoiesis, O_2 in bone marrow blood and, 618.
ESSEX, H. E. See COLFER and ESSEX, 27.
Esterase activity of blood plasma of dog, 746.
Estrone, response to, after ovariectomy, thyroid activity and, 760.
Exercise, effect of oxygen, altitude and, on breath-holding time, 142.
Explosive decompression injury, 607.
Eye changes from fluid injection into vitreous humor, 568.
EYSTER, J. A. E. and W. E. GILSON. Electrical characteristics of injuries to heart muscle, 572.

FACILITATION in man, 229.
Fat, dietary, effect of, on gastric evacuation, 461.
Fatigue of standing, 109.
—— produced by prolonged wakefulness, 253.
Femoral arterial pulse in hemorrhagic shock, 272.

FERRIS, B. G., JR., R. E. FORSTER, II, E. L. PILLION and W. R. CHRISTENSEN. Control of peripheral blood flow: responses in the human hand when extremities are warmed, 304.
Fibrinogen and coagulation factor in stored plasma, 405.
Fibrinolysin, anti-, in chick plasma, 661.
FINERTY, J. C. See DEMPSTER and FINERTY, 596.
FIZZELL, J. A. See GOLSETH and FIZZELL, 558.
FOLLIS, R. H., JR. The effect of adding boron to a potassium-deficient diet in the rat, 520.
Food intake and selection, effect of thyroxine on, 334.
—— selection, effect of environmental temperature on, 329.
FORSTER, R. E., II. See FERRIS, FORSTER, PILLION and CHRISTENSEN, 304.
FORSTER, R. P. An examination of some factors which alter glomerular activity in the rabbit kidney, 523.
—— and J. P. MAES. Effect of experimental neurogenic hypertension on renal blood flow and glomerular filtration rates in intact denervated kidneys of unanesthetized rabbits with adrenal glands demedullated, 534.
FRANKSTON, J. E. See ALBANESE, IRBY, FRANKSTON and LEIN, 389.
FREEDMAN, A. See HORVATH, FREEDMAN and GOLDEN, 99.
FRENCH, C. R. See BRITTON, PERTZOFF, FRENCH and KLINE, 7.
FRIEDMAN, C. L. See FRIEDMAN, POLLEY and FRIEDMAN, 340.
FRIEDMAN, M. See BYERS, FRIEDMAN and GARFIELD, 677.
FRIEDMAN, S. M., J. R. POLLEY and C. L. FRIEDMAN. The clearance of inulin and sodium p-aminohippurate in the rat, 340.
FUHRMAN, F. A. See TURNER and FUHRMAN, 325.

GARFIELD, M. M. See BYERS, FRIEDMAN and GARFIELD, 677.
Gastrectomy, loss of uropepsin from urine after, 415.
Gastric evacuation, effect of dietary fat on, 461.
—— lesions, suppression of, by anti-ulcer substances, 754.
GEMMILL, C. L. The effect of alloxan on muscle glycolysis, 613.
Genital structure and function, effect of hyperthyroidism on, 95.
GERARD, R. W. See TSCHIRGI and GERARD, 358.
GILSON, W. E. See EYSTER and GILSON, 572.
Glucose level of blood, effect of adrenal cortex extract on, 423.
Glycogen stores, liver, effect of discontinuous chronic anoxia on, 65.
Glycolysis, muscle, effect of alloxan on, 613.
GOLDEN, H. See HORVATH, FREEDMAN and GOLDEN, 99.
GOLSETH, J. G. and J. A. FIZZELL. Electromyographic studies on cats after section and suture of the sciatic nerve, 558.
GRANT, W. C. and W. S. ROOT. The relation of O_2 in bone marrow blood to posthemorrhagic erythropoiesis, 618.
GREELEY, P. O. The action of insulin as indicated by depancreatized herbivora, 46.
GREEN, H. D. See BOBB and GREEN, 697, 700.
——. See LIPTON, DENISON and GREEN, 693.
GROSSMAN, M. See MACK, GROSSMAN and KATZ, 654.
——. See WILBURNE, SCHLICHTER, GROSSMAN and CISNEROS, 504.
GUEST, M. M., R. C. MURPHY, S. R. BODNAR, A. G. WARE and W. H. SEEGERS. Physiological effects of a plasma protein: blood pressure, leucocyte concentration, smooth and cardiac muscle activity, 471.
——, A. G. WARE and W. H. SEEGERS. A quantitative study of antifibrinolysin in chick plasma: increase in antifibrinolysin activity during pteroylglutamic acid deficiency, 661.
——. See WARE, GUEST and SEEGERS, 58.
GUSTAVSON, R. G. See LANGHAM and GUSTAVSON, 760.
GUYTON, A. C. Analysis of respiratory patterns in laboratory animals, 78.
——. Measurement of the respiratory volumes of laboratory animals, 70.

HACK, M. H. See LEIMDORFER, ARANA and HACK, 588.

HALDI, J. and W. WYNN. Blood sugar levels and the behavior pattern of young healthy adults several hours after the ingestion of large amounts of sucrose, 263.

HAMILTON, A. S., W. M. PARKINS and F. WALTZER. A comparison of ten infusion fluids in the treatment of moderate and severe hemorrhage in animals, 641.

HAMILTON, W. F. See REMINGTON and HAMILTON, 292.

HARDENBERGH, E. See BRAUER and HARDENBERGH, 746.

HARPUDER, K., J. BYER and I. D. STEIN. The effect of intra-arterial injection of adrenalin upon blood flow of the human forearm, 181.

HARRIS, A. S. and W. P. MATLOCK. The effects of anoxemic anoxia on excitability, conduction and refractoriness of mammalian cardiac muscle, 493.

HAYNES, F. W. and L. DEXTER. Renin, hypertensinogen and hypertensinase concentration of blood of dogs during the development of hypertension by constriction of the renal artery, 190.

——, —— and R. E. SEIBEL. Renin content of renal venous blood of normal and hypertensive patients at rest, 198.

HEAGAN, B. S. See MILLER, HEAGAN and TAYLOR, 1.

Heart—blood flow in interauricular septal defect, 267.

——, evaluation of work of, 292.

——, failing, energy-rich phosphate supply of, 733.

—— muscle, effects of anoxemic anoxia on, 493.

—— —— injury, electrical signs of, 572.

—— size and function in semi-starvation and rehabilitation, 153.

Heart. *See* Cardiac.

Heat, radiant, sweat gland responses to, 365.

HELMER, O. M. and R. E. SHIPLEY. Recovery of a pressor principle from the blood plasma of cats given kidney extracts, 353.

Hemorrhage, infusion fluids in treatment of, 641.

HENSCHEL, A., O. MICKELSEN, H. L. TAYLOR and A. KEYS. Plasma volume and thiocyanate space in famine edema and recovery, 170.

——. See KEYS, HENSCHEL and TAYLOR, 153.

Heparin, effect of, on ischemic compression shock, 697.

Hepatectomy, blood uric acid and allantoin after nephrectomy and, 677.

Herbivora, depancreatized, action of insulin in, 46.

HERTZMAN, A. B., W. C. RANDALL and K. E. JOCHIM. Relations between cutaneous blood flow and blood content in the finger pad, forearm and forehead, 122.

HIMMELSTEIN, A. See COURNAND, MOTLEY, HIMMELSTEIN, DRESDALE and BALDWIN, 267.

——. See MOTLEY, COURNAND, WERKO, HIMMELSTEIN and DRESDALE, 315.

HINES, H. M. See KEMP, TUTTLE and HINES, 705.

Histamine excretion in parathyroidectomized dogs, 420.

HONORATO, R. The plasmatic cofactor of thromboplastin: its adsorption with prothrombin and fibrinogen, by alumina and tricalcium phosphate gels, 381.

—— and A. J. QUICK. The relation of fibrinogen to the coagulation factor which diminished in stored plasma, 405.

Hormone, lactogenic, specificity of, 394.

Hormones, growth and adrenocorticotropic, effect of, on urine in diabetes, 400.

——, steroid, bioassay of, 444.

HORVATH, S. M., A. FREEDMAN and H. GOLDEN. Acclimatization to extreme cold, 99.

HURST, V. and C. W. TURNER. The thyroid secretion rate of growing and mature mice, 686.

Hyperglycemia from action of adrenalin on central nervous system, 588.

Hypertension, relation of renin to, 190.

Hyperthyroidism, effect of, on genital structure and function, 95.

Hypophysial stalk extirpation, insulin tolerance and adrenal cortex after, 222.

Hypoxia, effect of adrenalin on blood sugar and, 321.

INFUSION, fluids, efficacy of, in burn shock, 428.

—— —— in treatment of hemorrhage, 641.

INGLE, D. J., M. C. PRESTRUD and J. E. NEZAMIS. The effect of insulin upon the level of blood amino acids in the eviscerated rat as related to the level of blood glucose, 682.
——, ——, —— and M. H. KUIZENGA. Effect of adrenal cortex extract upon the tolerance of the eviscerated rat for intravenously administered glucose, 423.
Injury, explosive decompression, 607.
Innervation, re-, spontaneous, in paretic muscles, 670.
Insulin, action of, in depancreatized herbivora, 46.
——, effect of, on blood amino acids, 682.
—— tolerance after hypophysial stalk extirpation, 222.
Intestinal absorption of saline, blood pressure and, 466.
Intestine, small, absorption of chloride from, carotid sinus and, 149.
IRBY, V. See ALBANESE, IRBY, FRANKSTON and LEIN, 389.
Ischemic compression shock, 693, 697, 700.
IVY, A. C. See ANNEGERS and IVY, 461.
——. See BUCHER and IVY, 415.

JOCHIM, K. E. See HERTZMAN, RANDALL and JOCHIM, 122.
JOHN, E. S. See PATT, SWIFT, TYREE and JOHN, 480.

KATZ, L. N. See MACK, GROSSMAN and KATZ, 654.
KAULBERSZ, J., T. L. PATTERSON, D. J. SANDWEISS and H. V. SALTZSTEIN. Alterations in urogastrone excretion produced by extirpation of various endocrine glands, 373.
KELLER, A. D. and C. G. BRECKENRIDGE. Retention of normal insulin tolerance and adrenal cortex after extirpation of the hypophysial stalk in the dog, 222.
KEMP, C. R., W. W. TUTTLE and H. M. HINES. Studies on the temperature characteristics, blood flow and activity in normal and denervated limbs of the dog, 705.
KEYS, A., A. HENSCHEL and H. L. TAYLOR. The size and function of the human heart at rest in semi-starvation and in subsequent rehabilitation, 153.
——. See HENSCHEL, MICKELSEN, TAYLOR and KEYS, 170.
Kidney, liver and, effect of adrenal cortex extract on, 580.
——, rabbit, glomerular activity in, 523.
——, rôle of, in resistance to ischemic compression, shock, 700.
KLINE, R. F. See BRITTON, PERTZOFF, FRENCH and KLINE, 7.
KOCHAKIAN, C. D. See VAIL and KOCHAKIAN, 580.
KORR, I. M. See DENSLOW, KORR and KREMS, 229.
KREMS, A. D. See DENSLOW, KORR and KREMS, 229.
KUIZENGA, M. H. See INGLE, PRESTRUD, NEZAMIS and KUIZENGA, 423.

LACTATION, induction of, during pregnancy, 394.
Lactogenic hormone, specificity of, 394.
LANGHAM, W. and R. G. GUSTAVSON. Effect of level of thyroid activity on response of ovariectomized rats to estrone, 760.
LARSEN, E. M. The fatigue of standing, 109.
LAYTON, A., M. W. MORGAN, JR. and J. M. D. OLMSTED. Refractive changes produced by injection of fluids into the vitreous humor, 568.
LEIMDORFER, A., R. ARANA and M. H. HACK. Hyperglycemia induced by the action of adrenalin on the central nervous system, 588.
LEIN, M. See ALBANESE, IRBY, FRANKSTON and LEIN, 389.
LEVINE, R. See COHN, LEVINE and STREICHER, 299.
LI, C. H. See BENNETT and LI, 400.
LIPTON, E. L., A. B. DENISON and H. D. GREEN. Ischemic compression shock; influence of body temperature and of temperature of traumatized tissues, 693.
Liver and kidney, effect of adrenal cortex extract on, 580.
—— glycogen stores, effect of discontinuous chronic anoxia on, 65.
——, removal of intravenously injected BSP from, 299.
Longevity, effects of caloric restriction on, 511.
Lungs, distensibility of, pulmonary congestion and, 654.

MACK, I., M. GROSSMAN and L. N. KATZ. The effect of pulmonary vascular congestion on the distensibility of the lungs, 654.
MAES, J. P. See FORSTER and MAES, 534.
MASON, G. D. See BRUNISH, MCWILLIAMS, MASON, ADAMS and ERSHOFF, 551.
MATLOCK, W. P. See HARRIS and MATLOCK, 493.
MCCARTHY, M. D. and W. M. PARKINS. Comparative effectiveness of albumin, globin, hemoglobin, gelatin, oxypolygelatin, saline, Ringer's, blood and plasma upon the survival of rats subjected to standardized scald burns, 428.
MCWILLIAMS, H. B. See BRUNISH, MCWILLIAMS, MASON, ADAMS and ERSHOFF, 551.
MEITES, J. and C. W. TURNER. The induction of lactation during pregnancy in rabbits and the specificity of the lactogenic hormone, 394.
MICKELSEN, O. See HENSCHEL, MICKELSEN, TAYLOR and KEYS, 170.
MILLER, R. A., B. S. HEAGAN and C. B. TAYLOR. The oxygen content of arterial blood in dogs breathing air at low barometric pressures, 1.
MISRAHY, G. and S. SALAMA. The alleged excretion of histamine in parathyroidectomized dogs, 420.
MORGAN, M. W., JR. See LAYTON, MORGAN and OLMSTED, 568.
MOSES, C. An experimental study of intramuscular pressure measurements, 488.
MOTLEY, H. L., A. COURNAND, L. WERKO, A. HIMMELSTEIN and D. DRESDALE. The influence of short periods of induced acute anoxia upon pulmonary artery pressures in man, 315.
——. See COURNAND, MOTLEY, HIMMELSTEIN, DRESDALE and BALDWIN, 267.
Motoneuron pools, human, facilitation in, 229.
MUNRO, F. L. See MUNRO and MUNRO, 409.
MUNRO, M. P. and F. L. MUNRO. The reversible inactivation of prothrombin: a factor responsible for its partial reactivation, 409.
MURPHY, R. C. See GUEST, MURPHY, BODNAR, WARE and SEEGERS, 471.
Muscle glycolysis, effect of alloxan on, 613.
——, heart, effects of anoxemic anoxia on, 493.
—— injury, heart, electrical signs of, 572.
——, skeletal, temperature, blood flow and activity in, 705.
Muscles, paretic, spontaneous re-innervation in, 670.
——, reflex responses of, to pressure, 229.
——, wrist, activity of, 596.
Muscular, intra-, pressure measurements, 488.

NARCOTICS, effects of, on nervous structures, 541.
Nephrectomy and hepatectomy, blood uric acid and allantoin after, 677.
Nerve action potential, effect of *Triturus* toxin on, 325.
——, sciatic, electromyographic studies on, 558.
Nervous structures, effects of narcotics on, 541.
—— system, central, hyperglycemic action of adrenalin on, 588.
Neural relationship to muscle, 229.
Neurones, spiral, action of adrenaline on, 37.
NEWTON, M. See SPEALMAN, NEWTON and POST, 628.
NEZAMIS, J. E. See INGLE, PRESTRUD and NEZAMIS, 682.
——. See INGLE, PRESTRUD, NEZAMIS and KUIZENGA, 423.
NORTHUP, D. W. See STICKNEY, NORTHUP and VAN LIERE, 466.
——. See VAN LIERE, STICKNEY and NORTHUP, 149.

OLMSTED, J. M. D. See LAYTON, MORGAN and OLMSTED, 568.
OSTER, R. H. and D. C. SMITH. The influence of adrenalin on blood sugar and resistance to hypoxia in the nembutalized cat, 321.
OTIS, A. B. See RAHN and OTIS, 202.
Ovarian dysfunction, pituitary and, in diabetes, 84.
Ovariectomy, thyroid activity and response to estrone after, 760.
Oxygen, altitude and exercise, effect of, on breath-holding time, 142.
—— content of arterial blood at low barometric pressures, 1.
—— in bone marrow blood, relation of, to erythropoieses, 618.

PARKINS, W. M. See HAMILTON, PARKINS and WALTZER, 641.
——. See McCARTHY and PARKINS, 428.
PATT, H. M., M. N. SWIFT, E. B. TYREE and E. S. JOHN. Adrenal response to total body x-radiation, 480.
PATTERSON, T. L. See KAULBERSZ, PATTERSON, SANDWEISS and SALTZSTEIN, 373.
PERTZOFF, V. A. See BRITTON, PERTZOFF, FRENCH and KLINE, 7.
Phosphate supply, energy-rich, of failing heart, 733.
PILHORN, H. R. See BISCHOFF and PILHORN, 444.
PILLION, E. L. See FERRIS, FORSTER, PILLION and CHRISTENSEN, 304.
Pituitary and ovarian dysfunction in diabetes, 84.
—— growth hormone, effect of, on urinary glucose and nitrogen in diabetes, 400.
Plasma, chick, antifibrinolysin in, 661.
—— factor, blood pressure and leucopenia, 471.
—— volume and thiocyanate space in famine edema, 170.
Plasmatic cofactor of thromboplastin, 381.
POLLEY, J. R. See FRIEDMAN, POLLEY and FRIEDMAN, 340.
POST, R. L. See SPEALMAN, NEWTON and POST, 628.
Posture, effect of temperature and, on blood volume, 628.
Potassium-deficient diet, effects of adding boron to, 520.
Pregnancy, induction of lactation during, 394.
PRESTRUD, M. C. See INGLE, PRESTRUD and NEZAMIS, 682.
——. See INGLE, PRESTRUD, NEZAMIS and KUIZENGA, 423.
Protein, plasma, physiological effects of, 471.
Prothrombin, reversible inactivation of, 409.
——, stability of, 58.
Pteroylglutamic acid deficiency, antifibrinolysin activity during, 661.
Pulmonary artery pressures, effect of acute anoxia on, 315.
—— blood flow in interauricular septal defect, 267.
—— congestion and distensibility of lungs, 654.
Pulse, femoral arterial, in hemorrhagic shock, 272.

QUICK, A. J. See HONORATO and QUICK, 405.

RAHN, H. and A. B. OTIS. Alveolar air during simulated flights to high altitudes, 202.
RALSTON, H. J., A. N. TAYLOR and H. W. ELLIOTT. Further studies on streamline blood flow in the arteries of the cat, 52.
RANDALL, W. C. Local sweat gland activity due to direct effects of radiant heat, 365.
——. See HERTZMAN, RANDALL and JOCHIM, 122.
RAYMOND, W. B. See RISLEY, RAYMOND and BARNES, 754.
Reflex mechanism, carotid sinus cardiovascular, 712, 722.
REMINGTON, J. W. and W. F. HAMILTON. The evaluation of the work of the heart, 292.
Renal circulation, blood pressure flow studies on, 534.
—— clearance of inulin and sodium p-aminohippurate, 340.
—— function in rabbit, 523.
—— humoral pressor mechanism in man, 198.
Renin content of renal venous blood, 198.
——, relation of, to hypertension, 190.
Reproductive capacity, effects of caloric restriction on, 511.
Respiratory failure, acute, carotid-mandibular reflex in, 358.
—— patterns in laboratory animals, 78.
—— volumes of laboratory animals, 70.
REYNOLDS, O. E. The effect of discontinuous chronic anoxia on liver glycogen stores, 65.
RICHTER, K. M. and C. A. WINTER. A quantitative study of the effect of hyperthyroidism on genital structure and function, 95.
RISLEY, E. A., W. B. RAYMOND and R. H. BARNES. The use of the Shay rat in studying anti-ulcer substances, 754.
RODBARD, S. Relationship between body temperature and blood sugar in the chicken, 67.

RODBARD, S. Studies on the recurrence of decompression sickness on re-ascent to high altitudes, 133.
——. The effect of oxygen, altitude and exercise on breath-holding time, 142.
ROOT, W. S. See GRANT and ROOT, 618.

SALAMA, S. See MISRAHY and SALAMA, 420.
SALTZSTEIN, H. C. See KAULBERSZ, PATTERSON, SANDWEISS and SALTZSTEIN, 373.
SANDWEISS, D. J. See KAULBERSZ, PATTERSON, SANDWEISS and SALTZSTEIN, 373.
SCHLICHTER, J. G. See WILBURNE, SCHLICHTER, GROSSMAN and CISNEROS, 504.
Sciatic nerve, electromyographic studies on, 558.
SEEGERS, W. H. See GUEST, MURPHY, BODNAR, WARE and SEEGERS, 471.
——. See GUEST, WARE and SEEGERS, 661.
——. See WARE, GUEST and SEEGERS, 58.
SEIBEL, R. E. See HAYNES, DEXTER and SEIBEL, 198.
SHIPLEY, E. G. and K. S. DANLEY. Pituitary and ovarian dysfunction in experimental diabetes, 84.
SHIPLEY, R. E. See HELMER and SHIPLEY, 353.
Shock, blood-borne vasotropic substances in, 239.
——, burn, efficacy of infusion fluids in, 428.
——, hemorrhagic, femoral arterial pulse in, 272.
——, ischemic compression, 693, 697, 700.
SIEGEL, P. S., I. E. ALEXANDER and H. L. STUCKEY. The change in specific gravity of the blood plasma of the rat during severe water privation, 729.
Skeletal muscle, temperature, blood flow and activity in, 705.
Skin, blood flow and content of, 122.
Sleep, fatigue due to deprivation of, 253.
SMITH, D. C. See OSTER and SMITH, 321.
SPEALMAN, C. R., M. NEWTON and R. L. POST. Influence of environmental temperature and posture on volume and composition of blood, 628.
Spinal neurones, action of adrenaline on, 37.
Starvation, semi-, and rehabilitation, heart size and function in, 153.
STAVRAKY, G. W. The action of adrenaline on spinal neurones sensitized by partial isolation, 37.
STEIN, I. D. See HARPUDER, BYER and STEIN, 181.
Steroid hormones, bioassay of, 444.
STICKNEY, J. C., D. W. NORTHUP and E. J. VAN LIERE. Systemic blood pressure as a factor in the absorption of saline from the small intestine, 466.
——. See VAN LIERE, STICKNEY and NORTHUP, 149.
STREICHER, D. See COHN, LEVINE and STREICHER, 299.
STUCKEY, H. L. See SIEGEL, ALEXANDER and STUCKEY, 729.
Sucrose, blood sugar levels after ingestion of, 263.
Sweating responses to radiant heat, 365.
SWIFT, M. N. See PATT, SWIFT, TYREE and JOHN, 480.

TAYLOR, A. N. See RALSTON, TAYLOR and ELLIOTT, 52.
TAYLOR, C. B. See MILLER, HEAGAN and TAYLOR, 1.
TAYLOR, H. L. See HENSCHEL, MICKELSEN, TAYLOR and KEYS, 170.
——. See KEYS, HENSCHEL and TAYLOR, 153.
Temperature and posture, effect of, on blood volume, 628.
——, blood, and peripheral vascular flow, 304.
——, blood flow and activity in skeletal muscle, 705.
——, body, and blood sugar in the chicken, 67.
——, ——, effect of, on ischemic compression shock, 693.
——, environmental, effect of, on food selection, 329.
Thiocyanate space, plasma volume and, in famine edema, 170.
Thromboplastin, plasmatic cofactor of, 381.
Thyroid activity and response to estrone after ovariectomy, 760.
—— secretion rate of mice, 686.
Thyroidectomy, para-, histamine excretion after, 42.
Thyroxine, effect of, on food selection and intake, 334.
Toxin, *Triturus*, effect of, on action potential, 325.

TSCHIRGI, R. D. and R. W. GERARD. The carotid-mandibular reflex in acute respiratory failure, 358.
TURNER, C. W. See HURST and TURNER, 686.
——. See MEITES and TURNER, 394.
TURNER, R. S. and F. A. FUHRMAN. Modification of the action potential of amphibian nerves by *Triturus* embryonic toxin, 325.
TUTTLE, W. W. See KEMP, TUTTLE and HINES, 705.
TYLER, D. B. The effect of amphetamine sulfate and some barbiturates on the fatigue produced by prolonged wakefulness, 253.
TYREE, E. B. See PATT, SWIFT, TYREE and JOHN, 480.

ULCER, ANTI-, substances, study of, in Shay rat, 754.
Uric acid, blood, after nephrectomy and hepatectomy, 677.
Urinary amino acid, effect of carbohydrate feeding on output of, 389.
—— glucose and nitrogen in diabetes, effect of hormones on, 400.
Urine, loss of uropepsin from, after gastrectomy, 415.
Urogastrone excretion after extirpation of endocrine glands, 373.
Uropepsin, loss of, from urine, after gastrectomy, 415.

VAIL, V. N. and C. D. KOCHAKIAN. The effect of adrenalectomy, adrenal cortical hormones, and testosterone propionate plus adrenal cortical extract on the "alkaline" and "acid" phosphatases of the liver and kidney of the rat, 580.
VAN HARREVELD, A. Effect of ether and pentobarbital on the polarisation state of central nervous elements, 541.
——. On the mechanism of the "spontaneous" re-innervation in paretic muscles, 670.
VAN LIERE, E. J., J. C. STICKNEY and D. W. NORTHUP. The effect of stimulation of the carotid sinus region on absorption of chloride from the small intestine, 149.
——. See STICKNEY, NORTHUP and VAN LIERE, 466.
VISSCHER, M. B. See BALL, BARNES and VISSCHER, 511.
Vitamin imbalance in reduced caloric intake, 551.
Vitreous humor, refractive changes from injection of fluid into, 568.
VONOTZKY, J. See DONHOFFER and VONOTZKY, 329, 334.

WAKEFULNESS, prolonged, 253.
WALKER, S. M. Observations on the action potentials induced by indirect stimulation of skeletal muscle in desoxycorticosterone acetate-treated rats on a low potassium diet, 451.
WALTZER, F. See HAMILTON, PARKINS and WALTZER, 641.
WANG, S. C. and H. L. BORISON. An analysis of the carotid sinus cardiovascular reflex mechanism, 712.
—— ——. Decussation of the pathways in the carotid sinus cardiovascular reflex: an example of the principle of convergence, 722.
WARE, A. G., M. M. GUEST and W. H. SEEGERS. Stability of prothrombin, 58.
——. See GUEST, MURPHY, BODNAR, WARE and SEEGERS, 471.
——. See GUEST, WARE and SEEGERS, 661.
Water privation, specific gravity of blood plasma in, 729.
WEBB, E. A. See ALEXANDER and WEBB, 272.
WERKO, L. See MOTLEY, COURNAND, WERKO, HIMMELSTEIN and DRESDALE, 315.
WILBURNE, W., J. G. SCHLICHTER, M. GROSSMAN and F. CISNEROS. The use of acetylcholine in the objective determination of circulation time and the fractionation of the vascular bed traversed, 504.
WINTER, C. A. See RICHTER and WINTER, 95.
WOLLENBERGER, A. On the energy-rich phosphate supply of the failing heart, 733.
Wrist muscles, activity of, 596.
WYNN, W. See HALDI and WYNN, 263.

X-RADIATION, total body, adrenal response to, 480.

ZWEIFACH, B. W. See CHAMBERS and ZWEIFACH, 239.